

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 14/705, 16/28, C12N 15/12	A1	(11) International Publication Number: WO 96/30406 (43) International Publication Date: 3 October 1996 (03.10.96)
(21) International Application Number: PCT/US95/04079 (22) International Filing Date: 30 March 1995 (30.03.95) (71) Applicant (for all designated States except US): HUMAN GENOME SCIENCES, INC. [US/US]; 9410 Key West Avenue, Rockville, MD 20850-3338 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): LI, Yi [CN/US]; 16125 Howard Landing Drive, Gaithersburg, MD 20878 (US). CAO, Liang [CN/GB]; 18B Suncrest Tower, Monmouth Terrace, Hong Kong (HK). NI, Jian [CN/US]; Apartment #204, 305 West Side Drive, Gaithersburg, MD 20878 (US). GENTZ, Reiner [DE/US]; 13404 Fairland Park Drive, Silver Spring, MD 20904 (US). BULT, Carol, J. [US/US]; 334 Park Hall South, Laurel, MD 20824 (US). SUTTON, Granger, G., III [US/US]; 6409 Snowman Court, Columbia, MD 21045 (US). ROSEN, Craig, A. [US/US]; 22400 Rolling Hill Road, Laytonsville, MD 20882 (US). (74) Agents: OLSTEIN, Elliot, M.; Carella, Byrne, Bain, Gilfillan, Cecchi, Stewart & Olstein, 6 Becker Farm Road, Roseland, NJ 07068 (US) et al.		(81) Designated States: AU, CA, CN, JP, KR, MX, NZ, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: HUMAN G-PROTEIN COUPLED RECEPTORS (57) Abstract Human G-protein coupled receptor polypeptides and DNA (RNA) encoding such polypeptides and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods for utilizing such polypeptides for identifying antagonists and agonists to such polypeptides and methods of using the agonists and antagonists therapeutically to treat conditions related to the underexpression and overexpression of the G-protein coupled receptor polypeptides, respectively. Also disclosed are diagnostic methods for detecting a mutation in the G-protein coupled receptor nucleic acid sequences and an altered level of the soluble form of the receptors.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	M	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

HUMAN G-PROTEIN COUPLED RECEPTORS

This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, the polypeptides of the present invention are human 7-transmembrane receptors. The transmembrane receptors are G-protein coupled receptors sometimes hereinafter referred to individually as GPR1, GPR2, GPR3 and GPR4. The invention also relates to inhibiting the action of such polypeptides.

It is well established that many medically significant biological processes are mediated by proteins participating in signal transduction pathways that involve G-proteins and/or second messengers, e.g., cAMP (Lefkowitz, Nature, 351:353-354 (1991)). Herein these proteins are referred to as proteins participating in pathways with G-proteins or PPG proteins. Some examples of these proteins include the GPC receptors, such as those for adrenergic agents and dopamine (Kobilka, B.K., et al., PNAS, 84:46-50 (1987); Kobilka, B.K., et al., Science, 238:650-656 (1987); Bunzow, J.R., et al., Nature, 336:783-787 (1988)), G-proteins themselves, effector proteins, e.g., phospholipase C, adenylyl cyclase, and phosphodiesterase, and actuator proteins, e.g., protein

kinase A and protein kinase C (Simon, M.I., et al., Science, 252:802-8 (1991)).

For example, in one form of signal transduction, the effect of hormone binding is activation of an enzyme, adenylate cyclase, inside the cell. Enzyme activation by hormones is dependent on the presence of the nucleotide GTP, and GTP also influences hormone binding. A G-protein connects the hormone receptors to adenylate cyclase. G-protein was shown to exchange GTP for bound GDP when activated by hormone receptors. The GTP-carrying form then binds to an activated adenylate cyclase. Hydrolysis of GTP to GDP, catalyzed by the G-protein itself, returns the G-protein to its basal, inactive form. Thus, the G-protein serves a dual role, as an intermediate that relays the signal from receptor to effector, and as a clock that controls the duration of the signal.

The membrane protein gene superfamily of G-protein coupled receptors has been characterized as having seven putative transmembrane domains. The domains are believed to represent transmembrane α -helices connected by extracellular or cytoplasmic loops. G-protein coupled receptors include a wide range of biologically active receptors, such as hormone, viral, growth factor and neuroreceptors.

G-protein coupled receptors have been characterized as including these seven conserved hydrophobic stretches of about 20 to 30 amino acids, connecting at least eight divergent hydrophilic loops. The G-protein family of coupled receptors includes dopamine receptors which bind to neuroleptic drugs used for treating psychotic and neurological disorders. Other examples of members of this family include calcitonin, adrenergic, endothelin, cAMP, adenosine, muscarinic, acetylcholine, serotonin, histamine, thrombin, kinin, follicle stimulating hormone, opsins and rhodopsins, odorant, cytomegalovirus receptors, etc.

Most G-protein coupled receptors have single conserved cysteine residues in each of the first two extracellular loops which form disulfide bonds that are believed to stabilize functional protein structure. The 7 transmembrane regions are designated as TM1, TM2, TM3, TM4, TM5, TM6, and TM7. TM3 is also implicated in signal transduction.

Phosphorylation and lipidation (palmitoylation or farnesylation) of cysteine residues can influence signal transduction of some G-protein coupled receptors. Most G-protein coupled receptors contain potential phosphorylation sites within the third cytoplasmic loop and/or the carboxy terminus. For several G-protein coupled receptors, such as the β -adrenoreceptor, phosphorylation by protein kinase A and/or specific receptor kinases mediates receptor desensitization.

The ligand binding sites of G-protein coupled receptors are believed to comprise a hydrophilic socket formed by several G-protein coupled receptors transmembrane domains, which socket is surrounded by hydrophobic residues of the G-protein coupled receptors. The hydrophilic side of each G-protein coupled receptor transmembrane helix is postulated to face inward and form the polar ligand binding site. TM3 has been implicated in several G-protein coupled receptors as having a ligand binding site, such as including the TM3 aspartate residue. Additionally, TM5 serines, a TM6 asparagine and TM6 or TM7 phenylalanines or tyrosines are also implicated in ligand binding.

G-protein coupled receptors can be intracellularly coupled by heterotrimeric G-proteins to various intracellular enzymes, ion channels and transporters (see, Johnson et al., Endoc., Rev., 10:317-331 (1989)). Different G-protein α -subunits preferentially stimulate particular effectors to modulate various biological functions in a cell. Phosphorylation of cytoplasmic residues of G-protein coupled receptors have been identified as an important mechanism for

the regulation of G-protein coupling of some G-protein coupled receptors.

G-protein coupled receptors are found in numerous sites within a mammalian host, for example, dopamine is a critical neurotransmitter in the central nervous system and is a G-protein coupled receptor ligand.

In accordance with one aspect of the present invention, there are provided novel polypeptides which have been putatively identified as G-protein coupled receptors and biologically active and diagnostically or therapeutically useful fragments and derivatives thereof. The polypeptides of the present invention are of human origin.

In accordance with another aspect of the present invention, there are provided isolated nucleic acid molecules encoding human G-protein coupled receptors, including mRNAs, DNAs, cDNAs, genomic DNA as well as antisense analogs thereof and biologically active and diagnostically or therapeutically useful fragments thereof.

In accordance with a further aspect of the present invention, there is provided a process for producing such polypeptides by recombinant techniques which comprises culturing recombinant prokaryotic and/or eukaryotic host cells, containing a human G-protein coupled receptor nucleic acid sequence, under conditions promoting expression of said protein and subsequent recovery of said protein.

In accordance with yet a further aspect of the present invention, there are provided antibodies against such polypeptides.

In accordance with another embodiment, there is provided a process for using the receptors to screen for receptor antagonists and/or agonists and/or receptor ligands.

In accordance with still another embodiment of the present invention there is provided a process of using such agonists to stimulate the G-protein coupled receptors for the

treatment of conditions related to the under-expression of the G-protein coupled receptors.

In accordance with another aspect of the present invention there is provided a process of using such antagonists for inhibiting the action of the G-protein coupled receptors for treating conditions associated with over-expression of the G-protein coupled receptors.

In accordance with yet another aspect of the present invention there is provided non-naturally occurring synthetic, isolated and/or recombinant G-protein coupled receptor polypeptides which are fragments, consensus fragments and/or sequences having conservative amino acid substitutions, of at least one transmembrane domain of the G-protein coupled receptor, such that G-protein coupled receptor polypeptides of the present invention may bind G-protein coupled receptor ligands, or which may also modulate, quantitatively or qualitatively, G-protein coupled receptor ligand binding.

In accordance with still another aspect of the present invention there are provided synthetic or recombinant G-protein coupled receptor polypeptides, conservative substitution and derivatives thereof, antibodies, anti-idiotypic antibodies, compositions and methods that can be useful as potential modulators of G-protein coupled receptor function, by binding to ligands or modulating ligand binding, due to their expected biological properties, which may be used in diagnostic, therapeutic and/or research applications.

It is still another object of the present invention to provide synthetic, isolated or recombinant polypeptides which are designed to inhibit or mimic various G-protein coupled receptors or fragments thereof, as receptor types and subtypes.

In accordance with yet a further aspect of the present invention, there is also provided diagnostic probes comprising nucleic acid molecules of sufficient length to

specifically hybridize to the G-protein coupled receptor nucleic acid sequences.

In accordance with yet another object of the present invention, there is provided a diagnostic assay for detecting a disease or susceptibility to a disease related to a mutation in a G-protein coupled receptor nucleic acid sequence.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

Figures 1-4 show the cDNA sequences and the corresponding deduced amino acid sequences of the four G-protein coupled receptors of the present invention, respectively. The standard one-letter abbreviation for amino acids are used. Sequencing was performed using a 373 Automated DNA sequencer (Applied Biosystems, Inc.). Sequencing accuracy is predicted to be greater than 97% accurate.

Figure 5 is an illustration of the amino acid homology between GPR1 (top line) and odorant receptor-like protein (bottom line).

Figure 6 illustrates the amino acid homology between GPR2 (top line) and the human Endothelial Differentiation Gene-1 (EDG-1) (bottom line).

Figure 7 illustrates the amino acid homology between GPR3 (top line) and a human G-protein coupled receptor open reading frame (ORF) (bottom line).

Figure 8 illustrates the amino acid homology between GPR4 and the chick orphan G-protein coupled receptor (bottom line).

In accordance with an aspect of the present invention, there are provided isolated nucleic acids (polynucleotides)

which encode for the mature polypeptides having the deduced amino acid sequences of Figures 1-4 (SEQ ID No. 2, 4, 6 and 8) or for the mature polypeptides encoded by the cDNAs of the clones deposited as ATCC Deposit No. 75981 (GPR1), 75983 (GPR2), 75976 (GPR3), 75979 (GPR4) on December 16, 1994.

A polynucleotide encoding the GPR1 polypeptide of the present invention may be isolated from the human breast. The polynucleotide encoding GPR1 was discovered in a cDNA library derived from human eight-week-old embryo. It is structurally related to the G protein-coupled receptor family. It contains an open reading frame encoding a protein of 296 amino acid residues. The protein exhibits the highest degree of homology to an odorant receptor-like protein with 66 % identity and 83 % similarity over a 216 amino acid stretch.

A polynucleotide encoding the GPR2 polypeptide of the present invention may be isolated from human liver, heart and leukocytes. The polynucleotide encoding GPR2 was discovered in a cDNA library derived from human adrenal gland tumor. It is structurally related to the G protein-coupled receptor family. It contains an open reading frame encoding a protein of 393 amino acid residues. The protein exhibits the highest degree of homology to human EDG-1 with 30 % identity and 52 % similarity over a 383 amino acid stretch. Potential ligands to GPR2 include but are not limited to anandamide, serotonin, adrenalin and noradrenalin.

A polynucleotide encoding the GPR3 polypeptide of the present invention may be isolated from human liver, kidney and pancreas. The polynucleotide encoding GPR3 was discovered in a cDNA library derived from human neutrophil. It is structurally related to the G protein-coupled receptor family. It contains an open reading frame encoding a protein of 293 amino acid residues. The protein exhibits the highest degree of homology to a human G-Protein Coupled Receptor open reading frame with 39 % identity and 61 % similarity over the entire amino acid sequence. Potential

ligands to GPR3 include but are not limited to platelet activating factor, thrombin, C5a and bradykinin.

A polynucleotide encoding the GPR4 polypeptide of the present invention may be found in human heart, spleen and leukocytes. The polynucleotide encoding GPR4 was discovered in a cDNA library derived from human twelve-week-old embryo. It is structurally related to the G-protein coupled receptor family. It contains an open reading frame encoding a protein of 344 amino acid residues. The protein exhibits the highest degree of homology to a chick orphan G-protein coupled receptor with 82 % identity and 91 % similarity over a 291 amino acid stretch. Potential ligands to GPR4 include but are not limited to thrombin, chemokine, and platelet activating factor.

The polynucleotides of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the mature polypeptides may be identical to the coding sequence shown in Figures 1-4 (SEQ ID No. 1, 3, 5 and 7) or that of the deposited clones or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same mature polypeptides as the DNA of Figures 1-4 (SEQ ID No. 1, 3, 5 and 7) or the deposited cDNAs.

The polynucleotides which encode for the mature polypeptides of Figures 1-4 (SEQ ID No. 2, 4, 6 and 8) or for the mature polypeptides encoded by the deposited cDNAs may include: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide.

Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptides having the deduced amino acid sequence of Figures 1-4 (SEQ ID No. 2, 4, 6 and 8) or the polypeptides encoded by the cDNAs of the deposited clones. The variants of the polynucleotides may be a naturally occurring allelic variant of the polynucleotides or a non-naturally occurring variant of the polynucleotides.

Thus, the present invention includes polynucleotides encoding the same mature polypeptides as shown in Figures 1-4 (SEQ ID No. 2, 4, 6 and 8) or the same mature polypeptides encoded by the cDNAs of the deposited clones as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptides of Figure 1-4 (SEQ ID No. 2, 4, 6 and 8) or the polypeptides encoded by the cDNAs of the deposited clones. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotides may have a coding sequence which is a naturally occurring allelic variant of the coding sequences shown in Figures 1-4 (SEQ ID No. 1, 3, 5 and 7) or of the coding sequences of the deposited clones. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptides.

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptides of the present invention. The marker sequence may be a hexa-

histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptides fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., et al., Cell, 37:767 (1984)).

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 50% and preferably 70% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode polypeptides which either retain substantially the same biological function or activity as the mature polypeptide encoded by the cDNAs of Figures 1-4 (SEQ ID No. 1, 3, 5 and 7) or the deposited cDNAs, i.e. function as a G-protein coupled receptor or retain the ability to bind the ligand for the receptor even though the polypeptides do not function as a G-protein coupled receptor, for example, soluble form of the receptors.

Alternatively, the polynucleotide may be a polynucleotide which has at least 20 bases, preferably 30 bases, and more preferably at least 50 bases which hybridize to a polynucleotide of the present invention and which has an identity thereto, as hereinabove described, and which does not retain activity. Such polynucleotides may be employed as probes for the polynucleotide of SEQ ID No. 1, for example, for recovery of the polynucleotide or as a diagnostic probe or as a PCR primer.

The deposit(s) referred to herein will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for purposes of Patent Procedure. These deposits are provided merely as convenience to those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112. The sequence of the polynucleotides contained in the deposited materials, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with any description of sequences herein. A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

The present invention further relates to G-protein coupled receptor polypeptides which have the deduced amino acid sequences of Figures 1-4 (SEQ ID No. 2, 4, 6 and 8) or which have the amino acid sequences encoded by the deposited cDNAs, as well as fragments, analogs and derivatives of such polypeptides.

The terms "fragment," "derivative" and "analog" when referring to the polypeptides of Figures 1-4 (SEQ ID No. 2, 4, 6 and 8) or that encoded by the deposited cDNAs, means a polypeptide which either retains substantially the same biological function or activity as such polypeptide, i.e. functions as a G-protein coupled receptor, or retains the ability to bind the ligand or the receptor even though the polypeptide does not function as a G-protein coupled receptor, for example, a soluble form of the receptor. An analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

The polypeptides of the present invention may be recombinant polypeptides, a natural polypeptides or synthetic polypeptides, preferably recombinant polypeptides.

The fragment, derivative or analog of the polypeptides of Figures 1-4 (SEQ ID No. 2, 4, 6 and 8) or that encoded by the deposited cDNAs may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide which is employed for purification of the mature polypeptide. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the

invention and the production of polypeptides of the invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the G-protein coupled receptor genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing polypeptides by recombinant techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative

examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli. lac or trp, the phage lambda P₁ promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Streptomyces, Salmonella typhimurium; fungal cells, such as yeast; insect cells such as Drosophila S2 and Spodoptera Sf9; animal cells such as CHO, COS or Bowes melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors

and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example. Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pbs, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); pTRC99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α -factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell

lysing agents, such methods are well known to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The G-protein coupled receptor polypeptides can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The polypeptides of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated.

Polypeptides of the invention may also include an initial methionine amino acid residue.

Fragments of the full length G-protein coupled receptor genes may be employed as a hybridization probe for a cDNA library to isolate the full length genes and to isolate other genes which have a high sequence similarity to the gene or similar biological activity. Probes of this type generally have at least 20 bases. Preferably, however, the probes have at least 30 bases and may contain, for example, 50 bases or more. In many cases, the probe has from 20 to 50 bases. The probe may also be used to identify a cDNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete G-protein coupled receptor gene including regulatory and promotor regions, exons, and introns. As an example of a screen comprises isolating the coding region of the G-protein coupled receptor gene by using the known DNA sequence to synthesize an oligonucleotide probe. Labeled oligonucleotides having a sequence complementary to that of the gene of the present invention are used to screen a library of human cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

The G-protein coupled receptors of the present invention may be employed in a process for screening for antagonists and/or agonists for the receptor.

In general, such screening procedures involve providing appropriate cells which express the receptor on the surface thereof. Such cells include cells from mammals, yeast, drosophila or E. Coli. In particular, a polynucleotide encoding the receptor of the present invention is employed to transfect cells to thereby express the respective G-protein coupled receptor. The expressed receptor is then contacted with a test compound to observe binding, stimulation or inhibition of a functional response.

One such screening procedure involves the use of melanophores which are transfected to express the respective G-protein coupled receptor of the present invention. Such a screening technique is described in PCT WO 92/01810 published February 6, 1992.

Thus, for example, such assay may be employed for screening for a receptor antagonist by contacting the melanophore cells which encode the G-protein coupled receptor with both the receptor ligand and a compound to be screened. Inhibition of the signal generated by the ligand indicates that a compound is a potential antagonist for the receptor, i.e., inhibits activation of the receptor.

The screen may be employed for determining an agonist by contacting such cells with compounds to be screened and determining whether such compound generates a signal, i.e., activates the receptor.

Other screening techniques include the use of cells which express the G-protein coupled receptor (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation, for example, as described in Science, volume 246, pages 181-296 (October 1989). For example, potential agonists or antagonists may be contacted with a cell which expresses the G-protein coupled receptor and a second messenger response, e.g. signal transduction or pH changes, may be measured to determine whether the potential agonist or antagonist is effective.

Another such screening technique involves introducing RNA encoding the G-protein coupled receptors into *Xenopus* oocytes to transiently express the receptor. The receptor oocytes may then be contacted in the case of antagonist screening with the receptor ligand and a compound to be screened, followed by detection of inhibition of a calcium signal.

Another screening technique involves expressing the G-protein coupled receptors in which the receptor is linked to a phospholipase C or D. As representative examples of such cells, there may be mentioned endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The screening for an antagonist or agonist may be accomplished as hereinabove described by detecting activation of the receptor or inhibition of activation of the receptor from the phospholipase second signal.

Another method involves screening for antagonists by determining inhibition of binding of labeled ligand to cells which have the receptor on the surface thereof. Such a method involves transfecting a eukaryotic cell with DNA encoding the G-protein coupled receptor such that the cell expresses the receptor on its surface and contacting the cell with a potential antagonist in the presence of a labeled form of a known ligand. The ligand can be labeled, e.g., by radioactivity. The amount of labeled ligand bound to the receptors is measured, e.g., by measuring radioactivity of the receptors. If the potential antagonist binds to the receptor as determined by a reduction of labeled ligand which binds to the receptors, the binding of labeled ligand to the receptor is inhibited.

G-protein coupled receptors are ubiquitous in the mammalian host and are responsible for many biological functions, including many pathologies. Accordingly, it is desirable to find compounds and drugs which stimulate the G-protein coupled receptors on the one hand and which can antagonize a G-protein coupled receptor on the other hand, when it is desirable to inhibit the G-protein coupled receptor.

For example, agonists for G-protein coupled receptors may be employed for therapeutic purposes, such as the treatment of asthma, Parkinson's disease, acute heart failure, hypotension, urinary retention, and osteoporosis.

In general, antagonists to the G-protein coupled receptors may be employed for a variety of therapeutic purposes, for example, for the treatment of hypertension, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy and psychotic and neurological disorders, including schizophrenia, manic excitement, depression, delirium, dementia or severe mental retardation, dyskinesias, such as Huntington's disease or Gilles dila Tourett's syndrome, among others. G-protein coupled receptor antagonists have also been useful in reversing endogenous anorexia and in the control of bulimia.

Examples of G-protein coupled receptor antagonists include an antibody, or in some cases an oligopeptide, which binds to the G-protein coupled receptors but does not elicit a second messenger response such that the activity of the G-protein coupled receptors is prevented. Antibodies include anti-idiotypic antibodies which recognize unique determinants generally associated with the antigen-binding site of an antibody. Potential antagonists also include proteins which are closely related to the ligand of the G-protein coupled receptors, i.e. a fragment of the ligand, which have lost biological function and when binding to the G-protein coupled receptors, elicit no response.

A potential antagonist also includes an antisense construct prepared through the use of antisense technology. Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes for the mature polypeptides of the present invention, is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple helix -see Lee et al., Nucl. Acids

Res., 6:3073 (1979); Cooney et al, Science, 241:456 (1988); and Dervan et al., Science, 251: 1360 (1991)), thereby preventing transcription and the production of G-protein coupled receptors. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of mRNA molecules into G-protein coupled receptors (antisense - Okano, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of G-protein coupled receptors.

Another potential antagonist is a small molecule which binds to the G-protein coupled receptor, making it inaccessible to ligands such that normal biological activity is prevented. Examples of small molecules include but are not limited to small peptides or peptide-like molecules.

Potential antagonists also include a soluble form of a G-protein coupled receptor, e.g. a fragment of the receptors, which binds to the ligand and prevents the ligand from interacting with membrane bound G-protein coupled receptors.

This invention additionally provides a method of treating an abnormal condition related to an excess of G-protein coupled receptor activity which comprises administering to a subject the antagonist as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to block binding of ligands to the G-protein coupled receptors and thereby alleviate the abnormal conditions.

The invention also provides a method of treating abnormal conditions related to an under-expression of G-protein coupled receptor activity which comprises administering to a subject a therapeutically effective amount of the agonist described above in combination with a pharmaceutically acceptable carrier, in an amount effective

to enhance binding of ligands to the G-protein coupled receptor and thereby alleviate the abnormal conditions.

The soluble form of the G-protein coupled receptors, antagonists and agonists may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the antagonist or agonist, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the pharmaceutical compositions may be employed in conjunction with other therapeutic compounds.

The pharmaceutical compositions may be administered in a convenient manner such as by the topical, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes. The pharmaceutical compositions are administered in an amount which is effective for treating and/or prophylaxis of the specific indication. In general, the pharmaceutical compositions will be administered in an amount of at least about 10 $\mu\text{g/kg}$ body weight and in most cases they will be administered in an amount not in excess of about 8 mg/Kg body weight per day. In most cases, the dosage is from about 10 $\mu\text{g/kg}$ to about 1 mg/kg body weight daily, taking into account the routes of administration, symptoms, etc.

The G-protein coupled receptor polypeptides, and antagonists or agonists which are polypeptides, may be employed in accordance with the present invention by expression of such polypeptides *in vivo*, which is often referred to as "gene therapy."

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) encoding a polypeptide *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding a polypeptide of the present invention.

Similarly, cells may be engineered *in vivo* for expression of a polypeptide *in vivo* by, for example, procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retrovirus, for example, an adenovirus which may be used to engineer cells *in vivo* after combination with a suitable delivery vehicle.

The present invention also provides a method for determining whether a ligand not known to be capable of binding to a G-protein coupled receptor can bind to such receptor which comprises contacting a mammalian cell which expresses a G-protein coupled receptor with the ligand under conditions permitting binding of ligands to the G-protein coupled receptor, detecting the presence of a ligand which

binds to the receptor and thereby determining whether the ligand binds to the G-protein coupled receptor.

This invention further provides a method of screening drugs to identify drugs which specifically interact with, and bind to, the human G-protein coupled receptors on the surface of a cell which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding the G-protein coupled receptor with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with and bind to a human G-protein coupled receptor of the present invention.

This invention also provides a method of detecting expression of the G-protein coupled receptor on the surface of a cell by detecting the presence of mRNA coding for a G-protein coupled receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human G-protein coupled receptor under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the G-protein coupled receptor by the cell.

This invention is also related to the use of the G-protein coupled receptor genes as part of a diagnostic assay for detecting diseases or susceptibility to diseases related to the presence of mutations in the G-protein coupled receptor genes. Such diseases, by way of example, are related to cell transformation, such as tumors and cancers.

Individuals carrying mutations in the human G-protein coupled receptor genes may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a patient's cells, such as from blood, urine, saliva, tissue biopsy and autopsy material. The genomic DNA

may be used directly for detection or may be amplified enzymatically by using PCR (Saiki et al., Nature, 324:163-166 (1986)) prior to analysis. RNA or cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid encoding the G-protein coupled receptor proteins can be used to identify and analyze G-protein coupled receptor mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled G-protein coupled receptor RNA or alternatively, radiolabeled G-protein coupled receptor antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

Sequence differences between the reference gene and genes having mutations may be revealed by the direct DNA sequencing method. In addition, cloned DNA segments may be employed as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequencing primer is used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled nucleotide or by automatic sequencing procedures with fluorescent-tags.

Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial

melting temperatures (see, e.g., Myers et al., Science, 230:1242 (1985)).

Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (e.g., Cotton et al., PNAS, USA, 85:4397-4401 (1985)).

Thus, the detection of a specific DNA sequence may be achieved by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes, (e.g., Restriction Fragment Length Polymorphisms (RFLP)) and Southern blotting of genomic DNA.

In addition to more conventional gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 500 or 600 bases; however, clones larger than 2,000 bp have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. FISH requires use of the clones from which the EST was derived, and the longer the better. For example, 2,000 bp is good, 4,000 is better, and more than 4,000 is probably not necessary to get good results a reasonable percentage of the time. For a review of this technique, see Verma et al., *Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian Inheritance in Man* (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the

affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies

(Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. Also, transgenic mice may be used to express humanized antibodies to immunogenic polypeptide products of this invention.

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the following examples certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μ g of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μ l of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μ g of DNA are digested with 20 to 250 units of enzyme in a larger

volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. et al., *Nucleic Acids Res.*, 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., et al., *Id.*, p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units to T4 DNA ligase ("ligase") per 0.5 µg of approximately equimolar amounts of the DNA fragments to be ligated.

Unless otherwise stated, transformation was performed as described in the method of Graham, F. and Van der Eb, A., *Virology*, 52:456-457 (1973).

Example 1

Bacterial Expression and Purification of GPR1

The DNA sequence encoding GPR1, ATCC # 75981, is initially amplified using PCR oligonucleotide primers corresponding to the 5' and 3' end sequences of the processed G-protein coupled receptor nucleotide sequence. Additional

nucleotides corresponding to the GPR1 nucleotide sequence are added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence 5' GACTAAAGCTTAATGAGTAGTGAAATGGTG 3' (SEQ ID No. 9) contains a HindIII restriction enzyme site followed by 19 nucleotides of G-protein coupled receptor coding sequence starting from the presumed terminal amino acid of the processed protein. The 3' sequence 5' GAACTTCTAGACCCTCAGGGTTGTAAATCAG 3' (SEQ ID No. 10) contains complementary sequences to an XbaI site and is followed by 20 nucleotides of GPR1 coding sequence. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc. Chatsworth, CA). pQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. pQE-9 is then digested with HindIII and XbaI. The amplified sequences are ligated into pQE-9 and are inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture is then used to transform E. coli strain M15/rep 4 (Qiagen, Inc.) by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.⁶⁰⁰) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-

thiogalacto pyranoside") is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation. The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized G-protein coupled receptor is purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). GPR1 is eluted from the column in 6 molar guanidine HCl pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100mM sodium phosphate, 10 mmolar glutathione (reduced) and 2 mmolar glutathione (oxidized). After incubation in this solution for 12 hours the protein is dialyzed to 10 mmolar sodium phosphate.

Example 2

Bacterial Expression and Purification of GPR2

The DNA sequence encoding GPR2, ATCC # 75983, is initially amplified using PCR oligonucleotide primers corresponding to the 5' and 3' end sequences of the processed GPR2 coding sequence. Additional nucleotides corresponding to GPR2 coding sequence are added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence 5' GACTAAAGCTTAATGAGGCCACATGGGCA 3' (SEQ ID No. 11) contains a HindIII restriction enzyme site followed by 19 nucleotides of GPR2 coding sequence starting from the presumed terminal amino acid of the processed protein. The 3' sequence 5' GAACTTCTAGACGAACTAGTGGATCCCCCGG 3' (SEQ ID No. 12) contains complementary sequences to an XbaI site and is followed by 21 nucleotides of GPR2 coding sequence. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc. Chatsworth,

CA). pQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter operat r (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. pQE-9 is then digested with HindIII and XbaI. The amplified sequences are ligated into pQE-9 and are inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture is then used to transform E. coli strain M15/rep 4 (Qiagen, Inc.) by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.⁶⁰⁰) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation. The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized GPR2 is purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). GPR2 is eluted from the column in 6 molar guanidine HCl pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100mM sodium

phosphate, 10 mmolar glutathione (reduced) and 2 mmolar glutathione (oxidized). After incubation in this solution for 12 hours the protein is dialyzed to 10 mmolar sodium phosphate.

Example 3

Bacterial Expression and Purification of GPR3

The DNA sequence encoding GPR3, ATCC # 75976, is initially amplified using PCR oligonucleotide primers corresponding to the 5' and 3' end sequences of the processed G-protein coupled receptor nucleotide sequence. Additional nucleotides corresponding to the GPR3 coding sequence are added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence 5' GACTAAAGCTTAATGGCGTCTTTCTCTGCT 3' (SEQ ID No. 13) contains a HindIII restriction enzyme site followed by 19 nucleotides of GPR3 coding sequence starting from the presumed terminal amino acid of the processed protein. The 3' sequence 5' GAACTTCTAGACTTCACACAGTTGTACTAT 3' (SEQ ID No. 14) contains complementary sequences to XbaI site and is followed by 19 nucleotides of GPR3 coding sequence. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc. Chatsworth, CA). pQE-9 encodes antibiotic resistance (Amp'), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. pQE-9 is then digested with XbaI and XbaI. The amplified sequences are ligated into pQE-9 and are inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture is then used to transform E. coli strain M15/rep 4 (Qiagen Inc.) by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers

kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.₆₀₀) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation. The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized GPR3 is purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). GPR3 is eluted from the column in 6 molar guanidine HCl pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100mM sodium phosphate, 10 mmolar glutathione (reduced) and 2 mmolar glutathione (oxidized). After incubation in this solution for 12 hours the protein is dialyzed to 10 mmolar sodium phosphate.

Example 4

Bacterial Expression and Purification of GPR4

The DNA sequence encoding GPR4, ATCC # 75979, is initially amplified using PCR oligonucleotide primers corresponding to the 5' and 3' end sequences of the processed GPR4 nucleotide sequence. Additional nucleotides corresponding to the GPR4 coding sequence are added to the 5'

and 3' sequences respectively. The 5' oligonucleotide primer has the sequence 5' GACTAAAGCTTAATGGTAAGCGTTAACAGC 3' (SEQ ID No. 15) contains a HindIII restriction enzyme site followed by 19 nucleotides of GPR4 coding sequence starting from the presumed terminal amino acid of the processed protein. The 3' sequence 5' GAACTTCTAGACTTCAGGCAGCAGATTCATT 3' (SEQ ID No. 16) contains complementary sequences to XbaI site and is followed by 20 nucleotides of GPR4 coding sequence. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc. Chatsworth, CA). pQE-9 encodes antibiotic resistance (Amp'), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. pQE-9 is then digested with HindIII and XbaI. The amplified sequences are ligated into pQE-9 and are inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture is then used to transform *E. coli* strain M15/rep 4 (Qiagen, Inc.) by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan'). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.₆₀₀) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene

expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation. The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized GPR4 is purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). GPR4 is eluted from the column in 6 molar guanidine HCl pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100mM sodium phosphate, 10 mmolar glutathione (reduced) and 2 mmolar glutathione (oxidized). After incubation in this solution for 12 hours the protein is dialyzed to 10 mmolar sodium phosphate.

Example 5

Expression of Recombinant GPR1 in COS cells

The expression of plasmid, GPR1 HA is derived from a vector pcDNA1/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3) E.coli replication origin, 4) CMV promoter followed by a polylinker region, a SV40 intron and polyadenylation site. A DNA fragment encoding the entire GPR1 precursor and a HA tag fused in frame to its 3' end is cloned into the polylinker region of the vector, therefore, the recombinant protein expression is directed under the CMV promoter. The HA tag correspond to an epitope derived from the influenza hemagglutinin protein as previously described (I. Wilson, H. Niman, R. Heighten, A. Cherenson, M. Connolly, and R. Lerner, 1984, Cell 37, 767). The infusion of HA tag to the target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

The plasmid construction strategy is described as follows:

The DNA sequence encoding GPR1, ATCC # 75981, is constructed by PCR using two primers: the 5' primer 5' GTCCAAGCTTGCCACCATGAGTAGTGAAATGGTG 3' (SEQ ID No. 17) contains a HindIII site followed by 18 nucleotides of GPR1 coding sequence starting from the initiation codon; the 3' sequence 5' CTAGCTCGAGTCAAGCGTAGTCTGGGACGTCGTATGGGTAGCAGG GTTGTAATCAGG 3' (SEQ ID No. 18) contains complementary sequences to an XhoI site, translation stop codon, HA tag and the last 15 nucleotides of the GPR1 coding sequence (not including the stop codon). Therefore, the PCR product contains a HindIII site, GPR1 coding sequence followed by HA tag fused in frame, a translation termination stop codon next to the HA tag, and an XhoI site. The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with HindIII and XhoI restriction enzymes and ligated. The ligation mixture is transformed into E. coli strain SURE (Stratagene Cloning Systems, La Jolla, CA) the transformed culture is plated on ampicillin media plates and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant GPR1, COS cells are transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the GPR1 HA protein is detected by radiolabeling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells are labelled for 8 hours with ³⁵S-cysteine two days post transfection. Culture media are then collected and cells are lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5). (Wilson, I. et al., Id. 37:767 (1984)). Both cell lysate and culture media are precipitated with a HA specific monoclonal

antibody. Proteins precipitated are analyzed on 15% SDS-PAGE gels.

Example 6

Expression of Recombinant GPR2 in COS cells

The expression of plasmid, GPR2 HA is derived from a vector pcDNA1/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3) E.coli replication origin, 4) CMV promoter followed by a polylinker region, a SV40 intron and polyadenylation site. A DNA fragment encoding the entire GPR2 precursor and a HA tag fused in frame to its 3' end is cloned into the polylinker region of the vector, therefore, the recombinant protein expression is directed under the CMV promoter. The HA tag correspond to an epitope derived from the influenza hemagglutinin protein as previously described (I. Wilson, H. Niman, R. Heighten, A Cherenson, M. Connolly, and R. Lerner, 1984, Cell 37, 767). The infusion of HA tag to the target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

The plasmid construction strategy is described as follows:

The DNA sequence encoding for GPR2, ATCC # 75983, is constructed by PCR using two primers: the 5' primer 5' GTCCAAGCTTGCCACCATGGTTGGTGGCACCTGG 3' (SEQ ID No. 19) contains an HindIII site followed by 18 nucleotides of GPR2 coding sequence starting from the initiation codon; the 3' sequence 5' CTAGCTCGAGTCAAGCGTAGTCTGGGACGTCGTATGGGTAGCAGTG GATCCCCCGTGC 3' (SEQ ID No. 20) contains complementary sequences to an XhoI site, translation stop codon, HA tag and the last 15 nucleotides of the GPR2 coding sequence (not including the stop codon). Therefore, the PCR product contains a HindIII site, GPR2 coding sequence followed by HA tag fused in frame, a translation termination stop codon next to the HA tag, and an XhoI site. The PCR amplified DNA

fragment and the vector, pcDNAI/Amp, are digested with HindIII and XhoI restriction enzymes and ligated. The ligation mixture is transformed into E. coli strain SURE (Stratagene Cloning Systems, La Jolla, CA) the transformed culture is plated on ampicillin media plates and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant GPR2, COS cells are transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the GPR2 HA protein is detected by radiolabelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells are labelled for 8 hours with ³⁵S-cysteine two days post transfection. Culture media are then collected and cells are lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5). (Wilson, I. et al., Id. 37:767 (1984)). Both cell lysate and culture media are precipitated with a HA specific monoclonal antibody. Proteins precipitated are analyzed on 15% SDS-PAGE gels.

Example 7

Expression of Recombinant GPR3 in COS cells

The expression of plasmid, GPR3 HA is derived from a vector pcDNAI/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3) E.coli replication origin, 4) CMV promoter followed by a polylinker region, a SV40 intron and polyadenylation site. A DNA fragment encoding the entire GPR3 precursor and a HA tag fused in frame to its 3' end is cloned into the polylinker region of the vector, therefore, the recombinant protein expression is directed under the CMV promoter. The HA tag

correspond to an epitope derived from the influenza hemagglutinin protein as previously described (I. Wilson, H. Niman, R. Heighten, A. Cherenson, M. Connolly, and R. Lerner, 1984, Cell 37, 767). The infusion of HA tag to the target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

The plasmid construction strategy is described as follows:

The DNA sequence encoding for GPR3, ATCC # 75976, is constructed by PCR using two primers: the 5' primer 5' GTCCAAGCTTGCCACCATGAACACCACAGTAATG 3' (SEQ ID No. 21) contains a HindIII site followed by 18 nucleotides of GPR3 coding sequence starting from the initiation codon; the 3' sequence 5' CTAGCTCGAGTCAAGCGTAGTCTGGGACGTCGTATGGGTAGCAAGG GATCCATACAAATGT 3' (SEQ ID No. 22) contains complementary sequences to an XhoI site, translation stop codon, HA tag and the last 18 nucleotides of the GPR3 coding sequence (not including the stop codon). Therefore, the PCR product contains a HindIII site, GPR3 coding sequence followed by HA tag fused in frame, a translation termination stop codon next to the HA tag, and an XhoI site. The PCR amplified DNA fragment and the vector, pCDNAI/Amp, are digested with HindIII and XhoI restriction enzymes and ligated. The ligation mixture is transformed into E. coli strain SURE (Stratagene Cloning Systems, La Jolla, CA) the transformed culture is plated on ampicillin media plates and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant GPR3, COS cells are transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the GPR3 HA protein is detected by radiolabelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies:

A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells are labelled for 8 hours with ^{35}S -cysteine two days post transfection. Culture media are then collected and cells are lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5) (Wilson, I. et al., Id. 37:767 (1984))). Both cell lysate and culture media are precipitated with a HA specific monoclonal antibody. Proteins precipitated are analyzed on 15% SDS-PAGE gels.

Example 8

Expression of Recombinant GPR4 in COS cells

The expression of plasmid, GPR4 HA is derived from a vector pcDNAI/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3) E.coli replication origin, 4) CMV promoter followed by a polylinker region, a SV40 intron and polyadenylation site. A DNA fragment encoding the entire GPR4 precursor and a HA tag fused in frame to its 3' end is cloned into the polylinker region of the vector, therefore, the recombinant protein expression is directed under the CMV promoter. The HA tag correspond to an epitope derived from the influenza hemagglutinin protein as previously described (I. Wilson, H. Niman, R. Heighten, A Cherenson, M. Connolly, and R. Lerner, 1984, Cell 37, 767). The infusion of HA tag to the target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

The plasmid construction strategy is described as follows:

The DNA sequence encoding for GPR4, ATCC # 75979, is constructed by PCR using two primers: the 5' primer 5' GTCCAAGCTTGCCACCATGGTAAGCGTTAACAGC 3' (SEQ ID No. 23) contains a HindIII site followed by 18 nucleotides of GPR4 coding sequence starting from the initiation codon; the 3' sequence 5' CTAGCTCGAGTCAAGCGTAGTCTGGGACGTCGTATGGGTAGCAGG

CAGCAGATTCATTGTC 3' (SEQ ID No. 24) contains complementary sequences to an XhoI site, translation stop codon, HA tag and the last 18 nucleotides of the GPR4 coding sequence (not including the stop codon). Therefore, the PCR product contains a HindIII site, GPR4 coding sequence followed by HA tag fused in frame, a translation termination stop codon next to the HA tag, and an XhoI site. The PCR amplified DNA fragment and the vector, pCDNAI/Amp, are digested with Hind III and XhoI restriction enzymes and ligated. The ligation mixture is transformed into E. coli strain SURE (Stratagene Cloning Systems, La Jolla, CA) the transformed culture is plated on ampicillin media plates and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant GPR4, COS cells are transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the GPR4 HA protein is detected by radiolabelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells are labelled for 8 hours with ³⁵S-cysteine two days post transfection. Culture media are then collected and cells are lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5) (Wilson, I. et al., Id. 37:767 (1984))). Both cell lysate and culture media are precipitated with a HA specific monoclonal antibody. Proteins precipitated are analyzed on 15% SDS-PAGE gels.

Example 9

Cloning and expression of GPR1 using the baculovirus expression system

The DNA sequence encoding the full length GPR1 protein, ATCC # 75981, is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene:

The 5' primer has the sequence 5' **CGGGATCCCTCCATGAG** TAGTGAAATGGTG 3' (SEQ ID No. 25) and contains a BamHI restriction enzyme site (in bold) followed by 4 nucleotides resembling an efficient signal for the initiation of translation in eukaryotic cells (Kozak, M., J. Mol. Biol., 196:947-950 (1987) which is just behind the first 18 nucleotides of the GPR1 gene (the initiation codon for translation "ATG" is underlined).

The 3' primer has the sequence 5' CGGGATCCCGCT CAGGGTTGTAAATCAGG 3' (SEQ ID No. 26) and contains the cleavage site for the BamHI restriction endonuclease and 18 nucleotides complementary to the 3' non-translated sequence of the GPR1 gene. The amplified sequences are isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment is then digested with the endonuclease BamHI and then purified again on a 1% agarose gel. This fragment is designated F2.

The vector pRG1 (modification of pVL941 vector, discussed below) is used for the expression of the GPR1 protein using the baculovirus expression system (for review see: Summers, M.D. and Smith, G.E. 1987, A manual of methods for baculovirus vectors and insect cell culture procedures, Texas Agricultural Experimental Station Bulletin No. 1555). This expression vector contains the strong polyhedrin promoter of the Autographa californica nuclear polyhedrosis virus (AcMNPV) followed by the recognition sites for the restriction endonuclease BamHI. The polyadenylation site of the simian virus (SV)40 is used for efficient polyadenylation. For an easy selection of recombinant virus the beta-galactosidase gene from E.coli is inserted in the same orientation as the polyhedrin promoter followed by the polyadenylation signal of the polyhedrin gene. The

polyhedrin sequences are flanked at both sides by viral sequences for the cell-mediated homologous recombination of cotransfected wild-type viral DNA. Many other baculovirus vectors could be used in place of pRG1 such as pAc373, pVL941 and pAcIM1 (Luckow, V.A. and Summers, M.D., Virology, 170:31-39).

The plasmid is digested with the restriction enzymes BamHI and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The DNA is then isolated from a 1% agarose gel using the commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.). This vector DNA is designated V2.

Fragment F2 and the dephosphorylated plasmid V2 are ligated with T4 DNA ligase. E.coli HB101 cells are then transformed and bacteria identified that contained the plasmid (pBacGPR1) with the GPR1 gene using the enzymes BamHI. The sequence of the cloned fragment is confirmed by DNA sequencing.

5 μ g of the plasmid pBacGPR1 is cotransfected with 1.0 μ g of a commercially available linearized baculovirus ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA.) using the lipofection method (Felgner et al. Proc. Natl. Acad. Sci. USA, 84:7413-7417 (1987)).

1 μ g of BaculoGold™ virus DNA and 5 μ g of the plasmid pBacGPR1 are mixed in a sterile well of a microtiter plate containing 50 μ l of serum free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards 10 μ l Lipofectin plus 90 μ l Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added dropwise to the Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is rocked back and forth to mix the newly added solution. The plate is then incubated for 5 hours at 27°C. After 5 hours the transfection solution is removed from the plate and 1 ml of

Grace's insect medium supplemented with 10% fetal calf serum is added. The plate is put back into an incubator and cultivation continued at 27°C for four days.

After four days the supernatant is collected and a plaque assay performed similar as described by Summers and Smith (supra). As a modification an agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used which allows an easy isolation of blue stained plaques. (A detailed description of a "plaque assay" can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10).

Four days after the serial dilution, the virus are added to the cells, blue stained plaques are picked with the tip of an Eppendorf pipette. The agar containing the recombinant viruses is then resuspended in an Eppendorf tube containing 200 µl of Grace's medium. The agar is removed by a brief centrifugation and the supernatant containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then stored at 4°C.

Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus V-GPR1 at a multiplicity of infection (MOI) of 2. Six hours later the medium is removed and replaced with SF900 II medium minus methionine and cysteine (Life Technologies Inc., Gaithersburg). 42 hours later 5 µCi of ³⁵S-methionine and 5 µCi ³⁵S cysteine (Amersham) are added. The cells are further incubated for 16 hours before they are harvested by centrifugation and the labelled proteins visualized by SDS-PAGE and autoradiography.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the

invention may be practiced otherwise than as particularly described.

SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- (i) APPLICANT: LI, ET AL.
- (ii) TITLE OF INVENTION: Human G-Protein Coupled Receptors
- (iii) NUMBER OF SEQUENCES: 26
- (iv) CORRESPONDENCE ADDRESS:
- (A) ADDRESSEE: CARELLA, BYRNE, BAIN, GILFILLAN, CECCHI, STEWART & OLSTEIN
- (B) STREET: 6 BECKER FARM ROAD
- (C) CITY: ROSELAND
- (D) STATE: NEW JERSEY
- (E) COUNTRY: USA
- (F) ZIP: 07068
- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: 3.5 INCH DISKETTE
- (B) COMPUTER: IBM PS/2
- (C) OPERATING SYSTEM: MS-DOS
- (D) SOFTWARE: WORD PERFECT 5.1
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER:
- (B) FILING DATE: Concurrently
- (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA
- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- (viii) ATTORNEY/AGENT INFORMATION:
- (A) NAME: FERRARO, GREGORY D.
- (B) REGISTRATION NUMBER: 36,134
- (C) REFERENCE/DOCKET NUMBER: 325800-270
- (ix) TELECOMMUNICATION INFORMATION:
- (A) TELEPHONE: 201-994-1700
- (B) TELEFAX: 201-994-1744
- (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS
- (A) LENGTH: 1713 BASE PAIRS
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

GGCAGGAGGT CATTCAACAT TTATTCAACC AAAAATACTA AGTCAGCTCT ATACAAACTA 60
ATGGAAGGAT ACAGCTATGC AAATATAGAA CACTAAAGTG TTACATGACA GATGTATGAG 120
TAGTGAAATG GTGAAAAATC AGACAATGGT CACAGAGTTC CTCCTACTGG GATTTCTCCT 180
GGGCCCAAGG ATTCAGATGC TCCTCTTTGG GCTCTTCTCC CTGTTCTATG TCTTCACCCT 240
GCTGGGGAAT GGGACCATCC TGGGGCTCAT CTCCTGGAC TCCAGACTCC ACACCCCAT 300
GTACTTCTTC CTCTCACACC TGGCCGTCGT CAACATCGCC TATGCCGCA ACACAGTGCC 360
CCAGATGCTG GTGAACCTCC TGCATCCAGC CAAGCCCATC TCCTTTGCTG GTTGATGAC 420
ACTAGACTTT CTCTTTTGA GTTTTGACA TACTGAATGC CTCCTGTTGG TGCTGATGTC 480
CTACGATCGG TACGTGGCCA TCTGCCACCC TCTCCGATAT TTCATCATCA TGACCTGGAA 540
AGTCGTGATC ACTCTGGGCA TCACCTTCCTG GACATGTGGC TCCCTCCTGG CTATGGTCCA 600
TGTGAGCCTC ATCTAAGAC TGCCCTTTTG TGGGCCTCGT GAAATCAACC ACTTCTTCTG 660
TGAAATCCTG TCTGCTCTCA GGCTGGCCTG TGCTGATACC TGGCTCAACC AGGTGGTCAT 720
CTTTGAAGCC TGCATGTTCA TCCTGGTGGG ACCACTCTGC CTGGTGCTGG TCTCCTACTC 780
ACACATCCTG GGGGGCATCC TGAGGATCCA GTCTGGGGAG GGCCGAGAA AGGCCTTCTC 840
CACCTGCTCC TCCCACCTCT GCGTAGTGGG ACTCTTCTTT GGSAGCGCCA TCGTCATGTA 900
CATGGCCCCC AAGTCCCGCC ATCCTGAGGA GCAGCAGAAG GTCTTTTTC TTATTTTACA 960
GTTCTTTTCA ACCCCGATGC TTAAACCCCC TGATTTACAA CCCTGAGGAA TGTAGAGGGT 1020
CAAGGGTGCC CTCGAGGAG ACCACTGTGC AARGRAAGTC ATTCTAAGG GGTGTGACAT 1080
TTGAACTGCC AGCCCCAGTT GCCCCGTGGA CTCCTGATGC CCAATTATTG CCTCAACCCA 1140
GAAAAGTTTA CTCCCTTTA ACTGTGCTTT ACTGACAGAA GGGCAAGCCT TCTCCCGTTT 1200
TTTGAGATA AAATTTTGA TGTGTTGCAA TCATTGGGTT TCTAGGAGAT GTGGTTTTAT 1260
CAGACAATTT TTTCTTTAT TTCACAATTA CTTTAATATC TGTAAATAA AGAATTATTT 1320
TAAATCATTT TCCAGTCCC AAAAGTTAAA TACAGGCCAC TACTTCTTT AACCAAATGA 1380
TATAGTTTGG CTCTGTGTCC CCACCCAAAT CTCATGTCAA ATTGTAATCC CCGCATGTCA 1440
GCGGAGGGAG CTGGTGGGAG GTGATTGGAT CATGGGGAGG GATTTCCCC TTGCTGTTCT 1500
GTTGATAGTG AACGAGTTCT CACGAAATCT GATGGTTTAA AAGTGCAGCA CTTCTCCCTT 1560
TGCTCTCTCT CTCTGCTGT GCCATGGTAA GACGTGCCTT GCTTCCCCTG GTGCTTCCGC 1620
CATGATGTGA CCTTCCTGA GGCCTCTCA GCCATGTGGA ACTGTGAGCC AATTAACTT 1680
CTTTTCTTTA GAAAAA AAAA AAAA AAAA 1713

```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 296 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Ser Ser Glu Met Val Lys Asn Gln Thr Met Val Thr Glu Phe
      5      10      15
Leu Leu Leu Gly Phe Leu Leu Gly Pro Arg Ile Gln Met Leu Leu
      20      25      30
Phe Gly Leu Phe Ser Leu Phe Tyr Val Phe Thr Leu Leu Gly Asn
      35      40      45
Gly Thr Ile Leu Gly Leu Ile Ser Leu Asp Ser Arg Leu His Thr
      50      55      60
Pro Met Tyr Phe Phe Leu Ser His Leu Ala Val Val Asn Ile Ala
      65      70      75
Tyr Ala Cys Asn Thr Val Pro Gln Met Leu Val Asn Leu Leu His
      80      85      90
Pro Ala Lys Pro Ile Ser Phe Ala Gly Cys Met Thr Leu Asp Phe

```

95	100	105
Leu Phe Leu Ser Phe Ala His Thr Glu Cys Leu Leu Leu Val Leu		
110	115	120
Met Ser Tyr Asp Arg Tyr Val Ala Ile Cys His Pro Leu Arg Tyr		
125	130	135
Phe Ile Ile Met Thr Trp Lys Val Cys Ile Thr Leu Gly Ile Thr		
140	145	150
Ser Trp Thr Cys Gly Ser Leu Leu Ala Met Val His Val Ser Leu		
155	160	165
Ile Leu Arg Leu Pro Phe Cys Gly Pro Arg Glu Ile Asn His Phe		
170	175	180
Phe Cys Glu Ile Leu Ser Val Leu Arg Leu Ala Cys Ala Asp Thr		
185	190	195
Trp Leu Asn Gln Val Val Ile Phe Glu Ala Cys Met Phe Ile Leu		
200	205	210
Val Gly Pro Leu Cys Leu Val Leu Val Ser Tyr Ser His Ile Leu		
215	220	225
Gly Gly Ile Leu Arg Ile Gln Ser Gly Glu Gly Arg Arg Lys Ala		
230	235	240
Phe Ser Thr Cys Ser Ser His Leu Cys Val Val Gly Leu Phe Phe		
245	250	255
Gly Ser Ala Ile Val Met Tyr Met Ala Pro Lys Ser Arg His Pro		
260	265	270
Glu Glu Gln Gln Lys Val Leu Phe Leu Ile Leu Gln Phe Leu Ser		
275	280	285
Thr Pro Met Leu Lys Pro Pro Asp Leu Gln Pro		
290	295	

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS
 (A) LENGTH: 2185 BASE PAIRS
 (B) TYPE: NUCLEIC ACID
 (C) STRANDEDNESS: SINGLE
 (D) TOPOLOGY: LINEAR

- (ii) MOLECULE TYPE: cDNA

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TCACTATAGG	GCGAATTGGG	TACGGGCCCC	CCCTCGAGGT	CGACGGTATC	GATAAGCTTG	60
ATATCGAATT	CGGCACGAGC	CGGGCTCGGA	GAGGTGACGG	AACCGGGGCT	GGTAGCATAG	120
TTTGATTTGA	TGATGGAGCC	AACACAGGGG	TTGGAGCTGG	TACCGGTGAA	GCTGAGGCTA	180
AAAAGGTTCC	TGGAGTAGAC	GATGGAGCCA	TAACTGGAAC	CGGAGTCTGT	GAATGAAGCC	240
AGGACAGGAG	CAGCACCTGG	CGATGGTGCC	AGGACCGGAA	GAGGAGCCAG	AGGAGGAGCT	300
GGAGAAGGAG	CCAGAATTGC	TGTCTGTGGA	GCCGCCATAG	GAGCCAGAGG	GGTGGCTAGA	360
GCCTGAGAAT	GCAGAAGATG	CTGGAGCCAG	AAGGGAAGCC	TGAGCTGGAG	CTGGATTTGG	420
TGCTGACGGA	AAAGGACTGG	CCAGAGCCGA	AGCTGGCACC	AGGGACAGGT	GAGCATTCTG	480
GGGCCACGGT	TGAGTTCAAC	CCACTGACTT	CAGGTGAAGG	ACTGTGGACC	AGCTTGAGAA	540
GAGGCCTCAC	CAGAGTGGGT	GTGGGGCATG	GGGGCTCGAG	CAGTACCCAG	AGTAGGTGTG	600
GGTAGCCCGG	CCAGGGGTTA	ACGTGGGGCG	TGGATTCAAC	ACAGCTTGGA	AGCCCAGAGC	660
TCGGAGGGCC	GGGTGCTTGG	GCCAATTGAG	GAACAGGAGT	CAGTCCATCC	CGAGGGGGTT	720
GTCTCACTAC	AATCTTCACA	CGCCTTTATT	ATTCACCATG	GTGGGTGGCA	CTGGTTAGC	780
AGCAAGCGGA	AGGCTGAGGC	CAGTAGGGGC	AGGGGTGTTA	CTGGGGGTG	AAGAAGCCAG	840
CACAGAGACA	GGGGTAGGGC	CAGGGGTCGG	GGCCACGGCC	TGGATGAGGC	CCACATGGGC	900
AGGCTGGCTG	ATGAGATGGT	GCTGCCCCCC	TGCTGACACG	AGGTGCACCA	CATTCTTTG	960

```

CAGCGGGCGG GCTGCCCCAC AGCAAGCTGG CGCACCTGGG CACCATCCAA AATACAGCTT 1020
GTTTCCCTGG ATTTGGAAGG TGAGAGGTTT GCTTCCCCCT CCATTAACCA CTGACGTTGT 1080
GCCAGTGAGA CTAACCTCTCC GCGCCAATCT GTCCGCGGGT GACCTCCTTC GCGGGCGTGG 1140
CCTACCTCTT CCTCATGTTC CACACTGTCC CCGCACAGCC CGACTTTCAC TTGAGGGCTG 1200
GTTCTGTGCG CAGGGCTTGC TGGACACAAA CCTCACTGCG TCGGTGGCCA CACTGTGGC 1260
CATCGCCGTG GAGCGGCACC GCAGTGTGAT GGCCGTGCAG CTGCACAGCC GCCTGCCCCG 1320
TGGCCGCGTG GTCATGCTCA TTGTGGGCGT GTGGGTGGCT GCCCTGGGCG TGGGGCTGCT 1380
GCCTGCCCCA TCCTGGCACT GCCTCTGTGC CCTGGACCGC TCCTCAGCA TGGCACCCT 1440
GCTCAGCCGC TCCTATTGG CCGTCTGGGC TCTGTCGAGC CTGCTGTCT TCCTGCTCAT 1500
GGTGGCTGTG TACACCCGCA TTTTCTTCTA CGTGGCGCGG CGAGTGCAGC GCATGGCAGA 1560
GCATGTCAGC TGCCACCCCC GCTACCGAGA GACCACGCTC AGCCTGGTCA AGACTGTTGT 1620
CATCATCTTG GGGGCGTTCG TGGTCTGCTG GACACCAGGC CAGGTGGTAC TGCTCCTGGA 1680
TGGTTTAGGC TGTGAGTCTT GCAATGTCCT GGCCTTAGAA AAGTACTTCC TACTGTTGGC 1740
CGAGCCAACC TCACTGGTCA ATGCTGCTGT GTACTCTTGC CGAGATGCTG AGATGCGCCG 1800
CACCTTCCGC CGCCTTCTCC TGCTGCGCGT GCCTCCGCCA GTCCACCCGC GAGTCTGTCC 1860
ACTATACATC CTCTGCCCAG GGAGGTGCCA GCACTCGCAT CATGCTTCCC GAGAACGGCC 1920
ACCCACTGAT GGACTCCACC CTTTAGCTAC CTTGAACACT AGCGGTACGC GGCAAGCAAC 1980
AAATCCACAG CCCCTGATGA CTTGTGGGTG CTCCTGGCTC AACCACACCT CGTGCCGAAT 2040
TCCTGCAGCC CGGGGGATCC ACTAGTCTA GAGCGGCGCC ACCGCGGTGG AGCTCCAGCT 2100
TTTGTTCCCT TTAGTGAGGG TTAATTCGA GCTTGGCGTA ATCATGGTCA TAGCTGTTTC 2160
CTGTGTGAAA TTGTTATCCG CTCAC 2185

```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 393 AMINO ACIDS
 (B) TYPE: AMINO ACID
 (C) STRANDEDNESS:
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Arg Pro Thr Trp Ala Gly Trp Leu Met Arg Trp Cys Cys Pro
      5                      10                      15
Pro Ala Asp Thr Arg Cys Thr Thr Phe Leu Cys Ser Gly Arg Ala
      20                      25                      30
Ala Pro Gln Gln Ala Gly Ala Pro Gly His His Pro Lys Tyr Ser
      35                      40                      45
Leu Phe Pro Trp Ile Trp Lys Val Arg Gly Leu Leu Pro Pro Pro
      50                      55                      60
Leu Thr Thr Asp Val Val Pro Val Arg Leu Thr Leu Arg Ala Asn
      65                      70                      75
Leu Ser Ala Ala Asp Leu Leu Arg Gly Arg Gly Leu Pro Leu Pro
      80                      85                      90
His Val Pro His Cys Pro Arg Thr Ala Arg Leu Ser Leu Glu Gly
      95                      100                     105
Trp Phe Leu Arg Gln Gly Leu Leu Asp Thr Asn Leu Thr Ala Ser
      110                     115                     120
Val Ala Thr Leu Leu Ala Ile Ala Val Glu Arg His Arg Ser Val
      125                     130                     135
Met Ala Val Gln Leu His Ser Arg Leu Pro Arg Gly Arg Val Val
      140                     145                     150
Met Leu Ile Val Gly Val Trp Val Ala Ala Leu Gly Leu Gly Leu
      155                     160                     165
Leu Pro Ala His Ser Trp His Cys Leu Cys Ala Leu Asp Arg Ser
      170                     175                     180

```

Ser	Arg	Met	Ala	Pro	Leu	Leu	Ser	Arg	Ser	Tyr	Leu	Ala	Val	Trp
				185					190					195
Ala	Leu	Ser	Ser	Leu	Leu	Val	Phe	Leu	Leu	Met	Val	Ala	Val	Tyr
				200					205					210
Thr	Arg	Ile	Phe	Phe	Tyr	Val	Arg	Arg	Arg	Val	Gln	Arg	Met	Ala
				215					220					225
Glu	His	Val	Ser	Cys	His	Pro	Arg	Tyr	Arg	Glu	Thr	Thr	Leu	Ser
				230					235					240
Leu	Val	Lys	Thr	Val	Val	Ile	Ile	Leu	Gly	Ala	Phe	Val	Val	Cys
				245					250					255
Trp	Thr	Pro	Gly	Gln	Val	Val	Leu	Leu	Leu	Asp	Gly	Leu	Gly	Cys
				260					265					270
Glu	Ser	Cys	Asn	Val	Leu	Ala	Leu	Glu	Lys	Tyr	Phe	Leu	Leu	Leu
				275					280					285
Ala	Glu	Pro	Thr	Ser	Leu	Val	Asn	Ala	Ala	Val	Tyr	Ser	Cys	Arg
				290					295					300
Asp	Ala	Glu	Met	Arg	Arg	Thr	Phe	Arg	Arg	Leu	Leu	Leu	Leu	Arg
				305					310					315
Val	Pro	Pro	Pro	Val	His	Pro	Arg	Val	Cys	Pro	Leu	Tyr	Ile	Leu
				320					325					330
Cys	Pro	Gly	Arg	Cys	Gln	His	Ser	His	His	Ala	Ser	Arg	Glu	Arg
				335					340					345
Pro	Pro	Thr	Asp	Gly	Leu	His	Pro	Leu	Ala	Thr	Leu	Asn	Tyr	Ser
				350					355					360
Gly	Thr	Arg	Gln	Ala	Thr	Asn	Pro	Gln	Pro	Leu	Met	Thr	Cys	Gly
				365					370					375
Cys	Ser	Trp	Leu	Asn	Pro	Thr	Ser	Cys	Arg	Ile	Pro	Ala	Ala	Arg
				380					385					390

Gly Ile His

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS
 (A) LENGTH: 1474 BASE PAIRS
 (B) TYPE: NUCLEIC ACID
 (C) STRANDEDNESS: SINGLE
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGGCACGAGC	ATAAGAAGAC	AGAGAGAACT	GAGTATCCTC	CCAAAGGTGA	CACTGGAAGC	60
AATGAACACC	ACAGTAATGC	AAGGCTTGAA	CAGATCTAAG	CGGTGCCCCA	AAGACACTCG	120
GATAGTACAG	CTGGTATTCC	CAGCCCTCTA	CACAGTGGTT	TTCTTGACCG	CAATCCTGCT	180
GAATACTTTG	GCTCTGTGGG	TGTTTGTTCA	CATCCCCAGC	TGGTCCACCT	TCATCATCTA	240
CCTCAAAAAC	ACTTTGGTGG	CCGACTTGAT	AATGACAGTG	ATGCTTCCTT	TCAAAATCCT	300
CTCTGACTCA	CACCTGGCAC	CCTGGCAGCT	CAGAGCTTTT	GTGTGTCGTT	TTTCTTCGGT	360
GATATTTTAT	GAGACCATGT	ATGTGGGCAT	AGTGCTGTTA	GGGCTCATAG	CCTTTGACAG	420
ATTCCTCAAG	ATCATCAGAC	CTTTGAGAAA	TATTTTCTTA	AAAAAACCTG	TTTGGGGAAA	480
AACGGTCTCA	ATCTTCATCT	GGTTCTTTTG	GTTCTTCATC	TCCCTGCCAA	ATATGATCTT	540
GAGCAACAAG	GAAGCAACAC	CATCGTCTGT	GAAAAAGTGT	GCTTCCTTAA	AGGGGCCTCT	600
GGGGCTGAAA	TGGCATCAAA	TGGTAAATAA	CATATGCCAG	TTTATTTTCT	GGACTGTTTT	660
TATCCTAATG	CTTGTGTTTT	ATGTGTTTAT	TGCAAAAAAG	TATATGATTC	TTATAGAAAG	720
TCCAAAAGTA	AGGACAGAAA	AAACAACAAA	AAGCTGGAAG	GCAAAGTATT	TGTTGTCGTG	780


```

GCTGTCTTCT TTGTGTGTTT TGCTCCATTT CATTTCGCCA GAGTTCCATA TACTCACAGT 840
CAAACCAACA ATAAGACTGA CTGTAGACTG CAAAATCAAC TGTTTATGTC TAAAGAAACA 900
ACTCTCTTTT TGGCAGCAAC TAACATTTGT ATGGATCCCT TAATATACAT ATTCTTATGT 960
AAAAAATTCA CAGAAAAGCT ACCATGTATG CAAGGGAGAA AGACCACAGC ATCAAGCCAA 1020
GAAAATCATA GCAGTCAGAC AGACAACATA ACCTTAGGCT GACAACTGTA CATAGGGGTA 1080
ACTTCTATTT ATTGATGAGA CTTCCGTAGA TAATGTGGAA ATCCAATTTA ACCAAGAAAA 1140
AAAGATTGGG GCAAATGCTC TCTTACATTT TATTATCCTG GTGTACAGAA AAGATTATAT 1200
AAAAATTTAA TCCACATAGA TCTATTCATA AGCTGAATGA ACCATTACTA AGAGAATGCA 1260
ACAGGATACA AATGGCCACT AGAGGTCATT ATTTGTTTCT TTCTTTCTTT TTTTTTTTTT 1320
AATTTCAGA GCATTTCACT TTAACATTTT GGAAAAGACT AAGGAGAAAC GTATATCCCT 1380
ACAAACCTCC CCTCCAAACA CTTTCTTACA TTCTTTTCCA CAATTCACAT AACACTACTG 1440
CTTTTGTGCC CCTTAAATGT AGATTTGTTG GCTG 1474

```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 293 AMINO ACIDS
 (B) TYPE: AMINO ACID
 (C) STRANDEDNESS:
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Met Asn Thr Thr Val Met Gln Gly Phe Asn Arg Ser Lys Arg Cys
      5                      10
Pro Lys Asp Thr Arg Ile Val Gln Leu Val Phe Pro Ala Leu Tyr
      20                      25
Thr Val Val Phe Leu Thr Gly Ile Leu Leu Asn Thr Leu Ala Leu
      35                      40
Trp Val Phe Val His Ile Pro Ser Ser Ser Thr Phe Ile Ile Tyr
      50                      55
Leu Lys Asn Thr Leu Val Ala Asp Leu Ile Met Thr Leu Met Leu
      65                      70
Pro Phe Lys Ile Leu Ser Asp Ser His Leu Ala Pro Trp Gln Leu
      80                      85
Arg Ala Phe Val Cys Arg Phe Ser Ser Val Ile Phe Tyr Glu Thr
      95                      100
Met Tyr Val Gly Ile Val Leu Leu Gly Leu Ile Ala Phe Asp Arg
     110                      115
Phe Leu Lys Ile Ile Arg Pro Leu Arg Asn Ile Phe Leu Lys Lys
     125                      130
Pro Val Trp Gly Lys Thr Val Ser Ile Phe Ile Trp Phe Phe Trp
     140                      145
Phe Phe Ile Ser Leu Pro Asn Met Ile Leu Ser Asn Lys Glu Ala
     155                      160
Thr Pro Ser Ser Val Lys Lys Cys Ala Ser Leu Lys Gly Pro Leu
     170                      175
Gly Leu Lys Trp His Gln Met Val Asn Asn Ile Cys Gln Phe Ile
     185                      190
Phe Trp Thr Val Phe Ile Leu Met Leu Val Phe Tyr Val Val Ile
     200                      205
Ala Lys Lys Tyr Met Ile Leu Ile Glu Ser Pro Lys Val Arg Thr
     215                      220
Glu Lys Thr Thr Lys Ser Trp Lys Ala Lys Tyr Leu Leu Ser Trp

```

	230		235		240
Leu Ser Ser Leu	Cys Val Leu Leu His	Phe Ile Ser Pro Glu	Phe		
	245		250		255
His Ile Leu Thr	Val Lys Pro Thr Ile	Arg Leu Thr Val Asp	Cys		
	260		265		270
Lys Ile Asn Cys	Leu Leu Leu Lys Lys	Gln Leu Ser Phe Trp	Gln		
	275		280		285
Gln Leu Thr Phe	Val Trp Ile Pro				
	290				

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS
 (A) LENGTH: 1301 BASE PAIRS
 (B) TYPE: NUCLEIC ACID
 (C) STRANDEDNESS: SINGLE
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

TTTTGGGTAT TTCTGAGAAA AAGGAAATAT TTATAAAACC ATCCAAAGAT CCAGATAATT      60
TGCAATAAAA TTGGAGGTTA TAGAGGTTAT AATCTGAATC CCAAAGGAGA CTGCAGCTGA      120
TGAAAGTGCT TCCAACTGA AAATTGGACG TGCCTTTACG ATGGTAAGCG TTAACAGCTC      180
CCACTGCTTC TATAATGACT CCTTTAAGTA CACTTTGTAT GGGTGCAATG TCAGCATGGT      240
GTTTGTGCTT GGGTTAATAT CCAATTGTGT TGCCATATAC ATTTTCATCT GCGTCCTCAA      300
AGTCCGAAAT GAAACTACAA CTTACATGAT TAACTTGGCA ATGTCAGACT TGCTTTTGTG      360
TTTTACTTTA CCCTTCAGGA TTTTACTT CACAACACGG AATTGGCCAT TTGGAGATTT      420
ACTTTGTAAG ATTTCTGTGA TGCTGTTTAA TACCAACATG TACGGAAGCA TTCTGTTCTT      480
AACCTGTATT AGTGTAGATC GATTCTGGC AATTGTCTAC CCATTTAAGT CAAAGACTCT      540
AAGAACCAAA AGAAATGCAA AGATTGTTTG ACATGGCGTG TGGTTAACTG TGATCGGAGG      600
AAGTGCACCC GCCGTTTITG TTCAGTCTAC CCACTCTCAG GGTAACAATG CCTCAGAAGC      660
CTGCTTTGAA AATTTTCCAG AAGCCACATG GAAACATAT CTCTCAAGGA TTGTAATTTT      720
CATCGAAATA GTGGGATTTT TTATTCCTCT AATTTTAAAT GTAACCTGTT CTAGTATGGT      780
GCTAAAAACT TTAACCAAAC CTGTTACATT AAGTAGAAGC AAAATAAACA AAACCTAAGG      840
TTTAAAAATG ATTTTGTAC ATTTGATCAT ATTCTGTTTC TGTTTTGTTC CTTACAATAT      900
CAATCTTATT TTATATTCTC TTGTGAGAAC ACAAACATTT GTTAATTGCT CAGTAGTGGC      960
AGCAGTAAGG ACAATGTACC CAATCACTCT CTGTATTGCT GTTCCAACT GTTGTTTTGA      1020
CCCTATAGTT TACTACTTTA CATCGGACAC AATTCAGAAT TCAATAAAAA TGAAAAACTG      1080
GTCTGTCAAG AGAAGTGACT TCAGATTCTC TGAAGTTCAT GGTGCAGAGA ATTTTATTCA      1140
GCATAACCTA CAGACCTTAA AAAGTAAGAT ATTTGACAAAT GAATCTGCTG CCTGAAATAA      1200
AACCATTAGG ACTCACTGGG ACAGAACTTT CAAGTTCCTT CAACTGTGAA AAGTGTCTTT      1260
TTGGACAAAC TATTTTTCCT CCTCCAAAAG AAATTAACAC A

```

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS
 (A) LENGTH: 344 AMINO ACIDS
 (B) TYPE: AMINO ACID
 (C) STRANDEDNESS:
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Val Ser Val Asn Ser Ser His Cys Phe Tyr Asn Asp Ser Phe

	5		10		15
Lys Tyr Thr Leu Tyr Gly Cys Met Phe		Ser Met Val Phe Val Leu			
20		25			30
Gly Leu Ile Ser Asn Cys Val Ala Ile Tyr Ile Phe Ile Cys Val		40			45
35		55			60
Leu Lys Val Arg Asn Glu Thr Thr Thr Tyr Met Ile Asn Leu Ala		70			75
50		85			90
Met Ser Asp Leu Leu Phe Val Phe Thr Leu Pro Phe Arg Ile Phe		100			105
65		115			120
Tyr Phe Thr Thr Arg Asn Trp Pro Phe Gly Asp Leu Leu Cys Lys		130			135
80		145			150
Ile Ser Val Met Leu Phe Tyr Thr Asn Met Tyr Gly Ser Ile Leu		160			165
95		175			180
Phe Leu Thr Cys Ile Ser Val Asp Arg Phe Leu Ala Ile Val Tyr		190			195
110		205			210
Pro Phe Lys Ser Lys Thr Leu Arg Thr Lys Arg Asn Ala Lys Ile		220			225
125		235			240
Val Cys Thr Gly Val Trp Leu Thr Val Ile Gly Gly Ser Ala Pro		250			255
140		265			270
Ala Val Phe Val Gln Ser Thr His Ser Gln Gly Asn Asn Ala Ser		280			285
155		295			300
Glu Ala Cys Phe Glu Asn Phe Pro Glu Ala Thr Trp Lys Thr Tyr		310			315
170		325			330
Leu Ser Arg Ile Val Ile Phe Ile Glu Ile Val Gly Phe Phe Ile		340			
185					
Pro Leu Ile Leu Asn Val Thr Cys Ser Ser Met Val Leu Lys Thr					
200					
Leu Thr Lys Pro Val Thr Leu Ser Arg Ser Lys Ile Asn Lys Thr					
215					
Lys Val Leu Lys Met Ile Phe Val His Leu Ile Ile Phe Cys Phe					
230					
Cys Phe Val Pro Tyr Asn Ile Asn Leu Ile Leu Tyr Ser Leu Val					
245					
Arg Thr Gln Thr Phe Val Asn Cys Ser Val Val Ala Ala Val Arg					
260					
Thr Met Tyr Pro Ile Thr Leu Cys Ile Ala Val Ser Asn Cys Cys					
275					
Phe Asp Pro Ile Val Tyr Tyr Phe Thr Ser Asp Thr Ile Gln Asn					
290					
Ser Ile Lys Met Lys Asn Trp Ser Val Arg Arg Ser Asp Phe Arg					
305					
Phe Ser Glu Val His Gly Ala Glu Asn Phe Ile Gln His Asn Leu					
320					
Gln Thr Leu Lys Ser Lys Ile Phe Asp Asn Glu Ser Ala Ala					
335					

- (2) INFORMATION FOR SEQ ID NO:9:
- (i) SEQUENCE CHARACTERISTICS
- (A) LENGTH: 30 BASE PAIRS
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

- (ii) MOLECULE TYPE: Oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
GACTAAAGCT TAATGAGTAG TGAAATGGTG 30
- (2) INFORMATION FOR SEQ ID NO:10:
- (i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 31 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
- (ii) MOLECULE TYPE: Oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
GAACTTCTAG ACCCTCAGGG TTGTAAATCA G 31
- (2) INFORMATION FOR SEQ ID NO:11:
- (i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 30 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
- (ii) MOLECULE TYPE: Oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
GACTAAAGCT TAATGAGGCC CACATGGGCA 30
- (2) INFORMATION FOR SEQ ID NO:12:
- (i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 32 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
- (ii) MOLECULE TYPE: Oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
GAACTTCTAG ACGAACTAGT GGATCCCCC GG 32
- (2) INFORMATION FOR SEQ ID NO:13:
- (i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 30 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
- (ii) MOLECULE TYPE: Oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
GACTAAAGCT TAATGGCGTC TTTCTCTGCT 30

- (2) INFORMATION FOR SEQ ID NO:14:
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 30 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
- (ii) MOLECULE TYPE: Oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
GAACTTCTAG ACTTCACACA GTTG TACTAT 30
- (2) INFORMATION FOR SEQ ID NO:15:
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 30 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
- (ii) MOLECULE TYPE: Oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
GACTAAAGCT TAATGGTAAG CGTTAACAGC 30
- (2) INFORMATION FOR SEQ ID NO:16:
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 31 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
- (ii) MOLECULE TYPE: Oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
GAACTTCTAG ACTTCAGGCA GCAGATTCAT T 31
- (2) INFORMATION FOR SEQ ID NO:17:
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 34 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
- (ii) MOLECULE TYPE: Oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:
GTCCAAGCTT GCCACCATGA GTAGTGAAAT GGTG 34
- (2) INFORMATION FOR SEQ ID NO:18:
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 58 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
CTAGCTCGAG TCAAGCGTAG TCTGGGACGT CGTATGGGTA GCAGGGTTGT AAATCAGG 58

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 34 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:
GTCCAAGCTT GCCACCATGG TTGGTGGCAC CTGG 34

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 58 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:
CTAGCTCGAG TCAAGCGTAG TCTGGGACGT CGTATGGGTA GCAGTGGATC CCCCCTGC 58

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 34 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
GTCCAAGCTT GCCACCATGA ACACCACAGT AATG 34

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 61 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CTAGCTCGAG TCAAGCGTAG TCTGGGACGT CGTATGGGTA GCAAGGGATC CATACAAATG 60
T 61

- (2) INFORMATION FOR SEQ ID NO:23:
- (i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 34 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
- (ii) MOLECULE TYPE: Oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
GTCCAAGCTT GCCACCATGG TAAGCGTTAA CAGC 34
- (2) INFORMATION FOR SEQ ID NO:24:
- (i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 61 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
- (ii) MOLECULE TYPE: Oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
CTAGCTCGAG TCAAGCGTAG TCTGGGACGT CGTATGGGTA GCAGGCAGCA GATTCAATTGT 60
C 61
- (2) INFORMATION FOR SEQ ID NO:25:
- (i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 30 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
- (ii) MOLECULE TYPE: Oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:
CGGGATCCCT CCATGAGTAG TGAAATGGTG 30
- (2) INFORMATION FOR SEQ ID NO:26:
- (i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 29 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
- (ii) MOLECULE TYPE: Oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:
CGGGATCCCG CTCAGGGTTG TAAATCAGG 29

WHAT IS CLAIMED IS:

1. An isolated polynucleotide selected from the group consisting of:
 - (a) a polynucleotide encoding the polypeptide as set forth in SEQ ID No. 2, SEQ ID No. 4, SEQ ID No. 6, and SEQ ID No. 8 or fragments, analogs or derivatives of said polypeptides;
 - (b) a polynucleotide capable of hybridizing to and which is at least 70% identical to the polynucleotide of (a); and
 - (c) a fragment of the polynucleotides of (a) or (b) wherein said fragment has at least 50 nucleotides.
2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.
3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.
4. The polynucleotide of Claim 1 wherein the polynucleotide is genomic DNA.
5. An isolated polynucleotide comprising a member selected from the group consisting of:
 - (a) the polynucleotide of Claim 2 encoding a polypeptide having the amino acid sequence encoded by the DNA contained in ATCC Deposit No. 75981;
 - (b) a polynucleotide capable of hybridizing to and which is at least 70% identical to the polynucleotide of (a); and
 - (c) a fragment of the polynucleotides of (a) or (b) wherein said fragment has at least 50 nucleotides.
6. An isolated polynucleotide comprising a member selected from the group consisting of:
 - (a) the polynucleotide of Claim 2 encoding a polypeptide having the amino acid sequence encoded by the DNA contained in ATCC Deposit No. 75983;

(b) a polynucleotide capable of hybridizing to and which is at least 70% identical to the polynucleotide of (a); and

(c) a fragment of the polynucleotides of (a) or (b) wherein said fragment has at least 50 nucleotides.

7. An isolated polynucleotide comprising a member selected from the group consisting of:

(a) the polynucleotide of Claim 2 encoding a polypeptide having the amino acid sequence encoded by the DNA contained in ATCC Deposit No. 75967;

(b) a polynucleotide capable of hybridizing to and which is at least 70% identical to the polynucleotide of (a); and

(c) a fragment of the polynucleotides of (a) or (b) wherein said fragment has at least 50 nucleotides.

8. An isolated polynucleotide comprising a member selected from the group consisting of:

(a) the polynucleotide of Claim 2 encoding a polypeptide having the amino acid sequence encoded by the DNA contained in ATCC Deposit No. 75979;

(b) a polynucleotide capable of hybridizing to and which is at least 70% identical to the polynucleotide of (a); and

(c) a fragment of the polynucleotides of (a) or (b) wherein said fragment has at least 50 nucleotides.

9. The polynucleotide of Claim 1 encoding the polypeptide having the amino acid sequence as shown in SEQ ID No. 2.

10. The polynucleotide of claim 9 having the coding sequence as shown in SEQ ID No. 1 from nucleotide 1 to nucleotide 1713.

11. The polynucleotide of Claim 1 encoding the polypeptide having the amino acid sequence as shown in SEQ ID No. 4.

12. The polynucleotide of claim 11 having the coding sequence as shown in SEQ ID No. 3 from nucleotide 1 to nucleotide 2185.
13. The polynucleotide of Claim 1 encoding the polypeptide having the amino acid sequence as shown in SEQ ID No. 6.
14. The polynucleotide of claim 13 having the coding sequence as shown in SEQ ID No. 5 from nucleotide 1 to nucleotide 1474.
15. The polynucleotide of Claim 1 encoding the polypeptide having the amino acid sequence as shown in SEQ ID No. 8.
16. The polynucleotide of claim 15 having the coding sequence as shown in SEQ ID No. 7 from nucleotide 1 to nucleotide 1301.
17. A vector containing the DNA of Claim 2.
18. A host cell genetically engineered with the vector of Claim 17.
19. A process for producing a polypeptide comprising: expressing from the host cell of Claim 18 the polypeptide encoded by said DNA.
20. A process for producing cells capable of expressing a polypeptide comprising genetically engineering cells with the vector of Claim 17.
21. An isolated DNA hybridizable to the DNA of Claim 2 and encoding a polypeptide having G-protein coupled receptor activity.
22. A polypeptide selected from the group consisting of: (i) a polypeptide having the deduced amino acid sequence of SEQ ID No. 2, SEQ ID No. 4, SEQ ID No. 6 and SEQ ID No. 8 and fragments, analogs and derivatives thereof, (ii) a polypeptide encoded by the cDNA of ATCC Deposit No. 75981, ATCC Deposit No. 75983, ATCC Deposit No. 75976 and ATCC Deposit No. 75979 and fragments, analogs and derivatives of said polypeptide.

23. An antibody against the polypeptide of claim 22.
24. A compound which activates the polypeptide of Claim 22.
25. A compound which inhibits activation of the polypeptide of claim 22.
26. A method for the treatment of a patient having need to activate a G-protein coupled receptor comprising: administering to the patient a therapeutically effective amount of the compound of Claim 24.
27. A method for the treatment of a patient having need to inhibit activation of a G-protein coupled receptor comprising: administering to the patient a therapeutically effective amount of the compound of Claim 25.
28. The polypeptide of Claim 22 wherein the polypeptide is a soluble fragment of the G-protein coupled receptor and is capable of binding a ligand for the receptor.
29. A process for identifying antagonists and agonists to the polypeptide of claim 22 comprising:
contacting a cell which expresses a G-protein coupled receptor with a known receptor ligand and a compound to be screened; and
determining if the compound inhibits or enhances activation of the receptor.
30. A process for determining whether a ligand not known to be capable of binding to the polypeptide of claim 22 can bind thereto comprising:
contacting a mammalian cell which expresses a G-protein coupled receptor with a potential ligand;
detecting the presence of the ligand which binds to the receptor; and
determining whether the ligand binds to the G-protein coupled receptor.
31. A method for diagnosing a disease or a susceptibility to a disease comprising:

detecting a mutation in the nucleic acid sequence encoding the polypeptide of claim 22 in a sample derived from a host.

32. A diagnostic process comprising:

analyzing for the presence of the polypeptide of claim 28 in a sample derived from a host.

1 / 18

FIG. 1A

10 30 50
GGCACGAGGTCAATCAACATTTATTCAACCAAAATACTAAGTCAGCTCTATACAAACTA
70 90 110
ATGGAAGGATACAGCTATGCAAAATATAGAACACACTAAAGTGTACATGACAGATGTATGAG
M S
130 150 170
TAGTGAATGGTGAAATCAGACAAATGGTCACAGAGTTCCTCTACTGGGATTTCTCCT
S E M V K N Q T M V T E F L L L G F L L
190 210 230
GGGCCCAAGGATTCAGATGCTCCTCTTTGGGCTCTTCTCCCTGTTCATGTCTTCACCCCT
G P R I Q M L L F G L F S L F Y V F T L
250 270 290
GCTGGGAATGGGACCATCCTGGGGCTCATCTCCTGACTCCAGACTCCACACCCCAT
L G N G T I L G L I S L D S R L H T P M
310 330 350
GTACTTCTTCCTCTCACACCTGCGCGTCGTCACATCGCCTATGCCTGCAACACAGTGCC
Y F F L S H L A V V N I A Y A C N T V P
370 390 410
CCAGATGCTGGTGAACTCCTGTCATCCAGCCAAAGCCCATCTCCTTTGCTGGTTGCATGAC
Q M L V N L L H P A K P I S F A G C M T
430 450 470
ACTAGACTTTCTCTTTTGAGTTTGCACATACTGAATGCCTCCTGTGGTGTGATGTC
L D F L F L S F A H T E C L L L V L M S
490 510 530
CTACGATCGGTACGTGGCCATCTGCCACCCCTCTCCGATATTTCAATCATCATGACCTGGAA
Y D R Y V A I C H P L R Y F I I M T W K
550 570 590
AGTCTGCATCACTCTGGGCATCACTTCTCTGGACATGTGGCTCCCTCTGGCTATGGTCCA

MATCH WITH FIG. 1B

SUBSTITUTE SHEET (RULE 28)

FIG. 1B

MATCH WITH FIG. 1A

V C I T L G I T S W T C G S L L A M V H
 610 630 650
 TGTGAGCCTCATCCTAAGACTGCCCTTTGTGGGCTCGTGAATCAACCACTTCTTCTG
 V S L I L R L P F C G P R E I N H F F C
 670 690 710
 TGAATCCTGTCTGTCTCAGGCTGGCCTGTGCTGATACCTGGCTCAACCAAGTGTGCAT
 E I L S V L R L A C A D T W L N Q V V I
 730 750 770
 CTTTGaAGCCTGCATGTTTCATCCTGGTGGGACCACCTCTGCCCTGGTGTCTCCTACTC
 F E A C M F I L V G P L C L V L V S Y S
 790 810 830
 ACACATCCTGGGGGCATCCTGAGGATCCAGTCTGGGAGGCCGCAGAAAGCCTTCTC
 H I L G G I L R I Q S G E G R R K A F S
 850 870 890
 CACCTGCTCCTCCACCTCTGCGTAGTGGGACTCTTCTTTGGSAGCGCCATCGTCATGTA
 T C S S H L C V V G L F F G S A I V M Y
 910 930 950
 CATGGCCCCCTAAGTCCCGCCATCCTGAGGAGCAGCAGAAGGTCCTTTTCTTATTAC
 M A P K S R H P E E Q Q K V L F L I L Q
 970 990 1010
 GTTCCTTTCAACCCCGATGCTTAAACCCCTGATTACAAACCTGAGGAATGTAGAGGGT
 F L S T P M L K P P D L Q P *
 1030 1050 1070
 CAAgggtGCCCTCCGAGGAGACCACTGTGCAARGRAAGTCATTCCTAAGGGGTGTGACAT
 1090 1110 1130

MATCH WITH FIG. 1C

FIG. 1C

MATCH WITH FIG. 1B

TTGAACTGCCAGCCCCAGTTGCCCCCGTGGACTCCTGATGCCCAATTATTGCCCCCAACCCA
1150 1170 1190
GAAAGTTTACTCCCCCTTTAACTGTGCTTTTACTGACAGAGGGCAAGCCTTCTCCCCGTTT
1210 1230 1250
TTTGACAGATAAAATTTTAGATGTGTGCAATCATTTGGGTTTCTAGGAGATGTGGTTTAT
1270 1290 1310
CAGACAAATTTTCTTTTATTTCACAAATTACTTTAAATATCTGTAAATAAAGAAATTATTT
1330 1350 1370
TAAATCATTTTCCCCAGTCCCCAAAAGTTAAATACAGGCCACTTACTTCTTTAACCAAATGA
1390 1410 1430
TATAGTTTGGCTCTGTGTCCCCACCCAAATCTCATGTCAAAATTGTAATCCCCCGCATGTCA
1450 1470 1490
GCGGAGGACCTGGTGGAGGTGATTGGATCATGGGGAGGGATTTCCTCCCTTGCTGTTCT
1510 1530 1550
GTTGATAGTGAACGAGTTCTCACGAAATCTGATGGTTTAAAGTGCAGCACTTCTCCCTT
1570 1590 1610
TGCTCTCTCTCTCCGTGTGCCATGGTAAGACGTGCCCTTGCTTCCCTTGCTTCCGC
1630 1650 1670
CATGATTGTACCTTTCTCTGAGGCCCTCTCCAGCCATGTGGAACTGTGAGCCCAATTAAACTT
1690 1710
CTTTTCTTTAGAAAAAATAAAAAAAAAA

SUBSTITUTE SHEET (RULE 28)

FIG. 2A

10 TCACTATAGGGCGAATTGGGTACGGGCCCCCTCGAGGTCGACGGTATCGATAAGCTTG 50
70 ATATCGAATTCGGCACGAGCCGGGCTCGGAGAGGTGACGGAACCGGGGCTGGTAGCATAG 110
130 TTTGATTTGATGATGGAGCCCAACACAGGGGTTGGAGCTGGTACCGGTGAAGCTGAGGCTA 170
190 AAAAGGTTCTGGAGTAGACGATGGAGCCATAACTGGAACCGGAGTCTGTGAATGAAGCC 230
250 AGGACAGGAGCAGCACCTGGCGATGGTGCCAGGACCGGAAGAGGAGCCAGAGGAGGCT 290
310 GGAGAAGGAGCCAGAAATTGCTGTCTGTGGAGCCGCCATAGGAGCCAGAGGGGTGGCTAGA 350
370 GCCTGAGAATGCAGAAGATGCTGGAGCCAGAAAGGGAAGCCTGAGCTGGAGCTGGATTGG 410
430 TGCTGACGGGAAAAGGACTGGCCAGAGCCGGAAGCTGGCACCCAGGACAGGTGAGCATTCCTG 470
490 GGGCCACGGTTGAGTTCAACCCACTGACTTCAGGTGAAGGACTGTGGACCCAGCTTGAGAA 530
550 GAGGCTCACCAGAGTGGGTGTGGGCGATGGGGCTCGAGCAGTACCCAGAGTAGGTGTG 590
610 GGTAGCCCGGCCAGGGGTTAACGTGGGGCGTGGATTCAACACAGCTTGGAAGCCAGAGC 650
670 TCGGAGGCCCGGGTGCTTGGGCCAATTGAGGAACAGGAGTCAGTCCATCCCAGGGGGTT 710
730 GTCTCACTACAAATCTTCACACGCCTTTATTTATTCACCATGGTTGTGTCACCTGGTTAGC 770
790 AGCAAGCGGAAGGCTGAGGCCAGTAGGGGCAGGGGTGTTACTGGGGGTCTGAAGAAGCCAG 830

MATCH WITH FIG. 2B

FIG. 2B

MATCH WITH FIG. 2A

850	CACAGACAGGGGTAGGGCCAGGGGTCGGGCCACGGCTGGATGAGGCCACATGGGC	870	M R P T W A
910	AGGCTGGCTGATGATGGTGCTGCCCCCTGCTGACACGAGGTGCACCATTCCTTTG	930	
	G W L M R W C C P P A D T R C T T F L C	950	
970	CAGCGCGGGCTGCCCCACAGCAAGCTGGCGCACCTGGGCACCATCCAAATACAGCTT	990	
	S G R A A P Q Q A G A P G H P K Y S L	1010	
1030	GTTTCCCTGGATTGGAAGGTGAGAGGTTTGCTTCCCCCTCCATTAACCACTGACGTTGT	1050	
	F P W I W K V R G L L P P P L T T D V V	1070	
1090	GCCAGTGAGACTAACTCTCCGGCCCAATCTGTCCGGGCTGACCTCCTTCGGGGCGTGG	1110	
	P V R L T L R A N L S A A D L L R G R G	1130	
1150	CCTACCTCTTCCATGTTCCACACTGTCCCCCGCACAGCCCCGACTTTCACCTGAGGGCTG	1170	
	L P L P H V P H C P R T A R L S L E G W	1190	
1210	GTTCCTGCGGCAGGGCTTGCTGGACACAAACCTCACTCGTGGTGCCACACTGCTGGC	1230	
	F L R Q G L L D T N L T A S V A T L L A	1250	
1270	CATCGCGTGAGCGGCACCGCAGTGATGGCGGTGACGTGCACAGCCGCTGCCCCG	1290	
	I A V E R H R S V M A V Q L H S R L P R	1310	
1330	TGGCCGCGTGTCATGCTCATTTGTGGCGGTGTGGGTGGCTGCCCTGGGGCTGGCT	1350	
	G R V V M L I V G V W V A A L G L G L L	1370	
1390		1410	
		1430	

MATCH WITH FIG. 2C

FIG. 2C

MATCH WITH FIG. 2B

GCCTGCCCACTCCTGGCACTGCCTCTGTGCCCTGGACCGCTCCTCAGCATGGCACCCCT
 P A H S W H C L C A L D R S S R M A P L 1450 1470 1490
 GCTCAGCCGCTCCTATTGGCCGCTCTGGGCTCTGTCGAGCCTGCTTGCTTCTCCTGCTCAT
 L S R S Y L A V W A L S S L L V F L L M 1510 1530 1550
 GGTGGCTGTGTACACCCGCAATTTCTTCTACGTGCGCGGCGAGTGCAGCGCATGGCAGA
 V A V Y T R I F Y V R R R V Q R M A E 1570 1590 1610
 GCATGTCACTGCCACCCCGCTACCGAGAGACACGCTCAGCCTGGTCAAGACTGTTGT
 H V S C H P R Y R E T T L S L V K T V V 1630 1650 1670
 CATCATCCTGGGGCGTTCGTGGTCTGTGGACACAGGCCAGGTGGTACTGCTCCTGGA
 I I L G A F V V C W T P G Q V V L L L D 1690 1710 1730
 TGGTTAGGCTGTGAGTCTGCAATGCTCCTGGCGTTAGAAAAGTACTTCTACTGTTGGC
 G L G C E S C N V L A L E K Y F L L L A 1750 1770 1790
 CGAGCCAACCTCACTGGTCAATGCTGTGTACTCTTGCCGAGATGCTGAGATGCGCGG
 E P T S L V N A A V Y S C R D A E M R R 1810 1830 1850
 CACCTTCGCGCGCTTCTCCTGCTGGCGGTGCTCCGCGAGTCCACCGGAGTCTGTCC
 T F R R L L L L R V P P P V H P R V C P

Match with FIG. 2D

7 / 18

FIG. 2D

MATCH WITH FIG. 2C

1870	1890	1910
ACTATACATCCTCTGCCCAGGAGGTGCCAGCACTCGCATCATGCTTCCCCGAGAACGGCC		
L Y I L C P G R C Q H S H A S R E R P		
1930	1950	1970
ACCCACTGATGGACTCCACCCCTTTAGCTACCTTGAACCTACAGCGGTACCGCGGCAAGCAAC		
P T D G L H P L A T L N Y S G T R Q A T		
1990	2010	2030
AAATCCACAGCCCCCTGATGACTTGTGGGTGCTCCTGGCTCAACCCAACTCGTGCCGAAT		
N P Q P L M T C G C S W L N P T S C R I		
2050	2070	2090
TCCTGCAGCCCCGGGGATCCACTAGTTCTAGAGCGGGCCACCCGGGTGGAGCTCCAGCT		
P A A R G I H *		
2110	2130	2150
TTTGTTCCCTTTAGTGAGGGTTAATTTCGAGCTTGGCGTAATCATGTGTCATAGCTGTTTC		
2170		
CTGTGTGAAATTGTTATCCGCTCAC		

FIG. 3A

10 30 50
CGGCACGAGCATAAGAAGACAGAGAGAACTGAGTATCCTCCCAAGGTGACACTGGAAGC 70 90 110
AATGAACACACACAGTAATGCAAGGCTTCAACAGATCTAAGCGGTGCCCAAGACACTCG 130 150 170
M N T T V M Q G F N R S K R C P K D T R
GATAGTACAGCTGGTATTCACGCGCTCTACACAGTGGTTTCTTGACCGGAATCCTGCT 190 210 230
I V Q L V F P A L Y T V V F L T G I L L
GAATACTTTGGCTCTGTGGGTGTTGTTTCACATCCCCAGCTCCTCCACCTTCATCATCTA 250 270 290
N T L A L W V F V H I P S S S T F I I Y
CCTCAAAAACACTTTGGTGGCCGACTTGATAATGACACTCATGCTTCCTTTCAAAATCCT 310 330 350
L K N T L V A D L I M T L M L P F K I L
CTCTGACTCACACCTGGCACCCCTGGCAGCTCAGAGCTTTTGTGTGTCGTTTCTTCGGT 370 390 410
S D S H L A P W Q L R A F V C R F S S V
GATATTTATGAGACCATGTATGTGGCATCGTGTGTAGGGCTCATAGCCTTTGACAG 430 450 470
I F Y E T M Y V G I V L L G L I A F D R
ATTCCTCAAGATCATCAGACCTTTGAGAAATATTTTCTAAAAAACCTGTTGGGAAA 490 510 530
F L K I I R P L R N I F L K K P V W G K
AACGGTCTCAATCTTCATCTGGTCTTTTGGTCTTCATCTCCCTGCCAAATATGATCTT 550 570 590
T V S I F I W F F F F I S L P N M I L

MATCH WITH FIG. 3B

FIG. 3B

MATCH WITH FIG. 3A

GAGCAACAAGGAAGCAACACCATCGTCTGTGAAAAAGTGTGCTTCCTTAAAGGGGCCTCT
 S N K E A T P S S V K K C A S L K G P L
 610 630 650
 GGGGCTGAAATGGCATCAAAATGGTAAATAACATATGCCAGTTTATTTCTGGACTGTTT
 G L K W H Q M V N N I C Q F I F W T V F
 670 690 710
 TATCCTAATGCTTGTGTTTATGTGGTTATTCGCAAAAAGTATATGATTCCTTATAGAAAG
 I L M L V F Y V V I A K K Y M I L I E S
 730 750 770
 TCCAAAAGTAAGGACAGAAAAACAACAAGCTGGAGGCAAGTATTTGTGTCGTG
 P K V R T E K T T K S W K A K Y L L S W
 790 810 830
 GCTGTCTTCTTTGTGTGTTTGTCTCCATTTTCATTTCCGCCAGAGTTCCCATATACTCACAGT
 L S S L C V L L H F I S P E F H I L T V
 850 870 890
 CAAACCAACAATAAGACTGACTGTAGACTGCAGAAATCAACTGTTTATGCTAAAGAAACA
 K P T I R L T V D C K I N C L L L K K Q
 910 930 950
 ACTCTCTTTTGGCAGCAACTAACATTTGTATGGATCCCTTAATATACATATCTTATGT
 L S F W Q Q L T F V W I P *
 970 990 1010
 AAAAAATTCACAGAAAAGCTACCATGTATGCAAGGGAGAAAGACCACAGCATCAAGCCAA
 1030 1050 1070
 GAAATCATAGCAGTCAGACAGACAACATAACCTTAGGCTGACAACTGTACATAGGGGTA

MATCH WITH FIG. 3C

FIG. 3C

Match with FIG. 3B

1090	1110	1130
ACTTCATTATTGATGAGACTTCCGTAGATAATGTGGAATCCAATTTAAACCAAGAAAA		
1150	1170	1190
AAAGATTGGGGCAAATGCTCTCTTACATTTTATTATCCTGGGTACAGAAAAGATTATAT		
1210	1230	1250
AAAATTAAATCCACATAGATCTATTTCATAAAGCTGAATGAACCATTAAGAGAAATGCA		
1270	1290	1310
ACAGGATACAAAATGGCCACTAGAGGTCATTATTCTTCTTCTTTCTTTTCTTTT		
1330	1350	1370
AATTTCAGAGCATTTTCACTTTTAACATTTTGGAAAAGACTAAGGAGAAAACGTATATCCCT		
1390	1410	1430
ACAAACCTCCCCCTCCAAACACCTTCTTACATTTCTTTTCCACAATTCACATAACACTACTG		
1450	1470	
CTTTTGTGCCCCCTTAAATGTAGATTGTTGGCTG		

FIG. 4A

```

10      TTTTGGGTATTTCTGAGAAAAGGAAATATTTATAAAACCATCCAAAGATCCAGATAATT      50
70      TGCAAAATAAATTGGAGGTTATAGAGGTTATAATCTGAATCCCAAAGGAGACTGCAGCTGA      110
130     TGAAAGTGCTTCCAAACTGAAATTTGGACGTGCCTTTACGATGGTAAGCGTTAACAGCTC      170
                                     M V S V N S S
190     CCACTGCTTCTATAATGACTCCTTTAAGTACACTTTGTATGGGTGCATGTTTCAGCATGGT      230
H C F Y N D S F K Y T L Y G C M F S M V
250     GTTGTGCTTGGGTTAATATCCAATTGTGTGCCATATACATTTTCATCTGCGTCCTCAA      290
F V L G L I S N C V A I Y I F I C V L K
310     AGTCCGAAATGAAACTACAACCTTACATGATTAACCTTGGCAATGTCAGACTTGCTTTTGT      350
V R N E T T T Y M I N L A M S D L L F V
370     TTTTACTTTACCCCTTCAGGATTTTCTTACTTCACAACACCGAATTGGCCATTGGAGATT      410
F T L P F R I F Y F T T R N W P F G D L
430     ACTTTGTAAGATTTCTGTGATGCTGTTTATACCAACATGTACGGAAGCATCTCTTCTT      470
L C K I S V M L F Y T N M Y G S I L F L
490     AACCTGTATTAGTGTAGATCGATTCTTCTGGCAATTGTCTACCCATTAAAGTCAAAGACTCT      530
T C I S V D R F L A I V Y P F K S K T L

```

MATCH WITH FIG. 4B

FIG. 4B

MATCH WITH FIG. 4A

550 AAGAACCAAAAGAAATGCAAGATTGTTGCACTGGCGTGGTAACTGTGATCGGAGG 590
 R T K R N A K I V C T G V W L T V I G G
 610 AAGTGCACCCGCGTTTGTTCAGTCTACCCACTCTCAGGTAACAATGCCCTCAGAAGC 650
 S A P A V F V Q S T H S Q G N N A S E A
 670 CTGCTTTGAAATTTCCAGAAGCCACATGGAAACATATCTCTCAAGGATTGTAATTT 710
 C F E N F P E A T W K T Y L S R I V I F
 730 CATCGAAATAGTGGATTTTTATTCCTCTAATTTTAAATGTAACCTGTCTAGTAGTGGT 770
 I E I V G F F I P L I L N V T C S S M V
 790 GCTAAAACTTTAACCACCTGTTACATTAGTAGAAGCAAAATAAACAATAAGGT 830
 L K T L T K P V T L S R S K I N K T K V
 850 TTTAAAAATGATTTTGTACATTGTGATCATATTCTGTTTCTGTTTGTTCCTTACAATAT 890
 L K M I F V H L I I F C F C F V P Y N I
 910 CAATCTTATTATTCCTCTGTGAGAACACAAACATTTGTTAATTGCTCAGTAGTGGC 950
 N L I L Y S L V R T Q T F V N C S V V A

MATCH WITH FIG. 4C

13 / 18

FIG. 4C

Match with FIG. 4B

```

970          990          1010
AGCAGTAAGGACAATGTACCCCAATCACTCTCTGTATTTGCTGTTCCAACTGTTGTTTGA
A V R T M Y P I T L C I A V S N C C F D
1030          1050          1070
CCCTATAGTTTACTACTTTACATCGGACACAAATTCAGAATTCATAAATAAGAAACTG
P I V Y Y F T S D T I Q N S I K M K N W
1090          1110          1130
GTCGTGTCAGGAGAAGTGACTTCAGATTCTCTGAAGTTCATGGTGCAGAGAATTTTATCA
S V R R S D F R F S E V H G A E N F I Q
1150          1170          1190
GCATAACCTACAGACCTTAAAGTAAGATATTGACAAATGAATCTGCTGCCCTGAAATAA
H N L Q T L K S K I F D N E S A A *
1210          1230          1250
AACCATTAGGACTCACTGGGACAGAACTTTCAAGTTCCTTCAACTGTGAAAAGTGCTCTT
1270          1290
TTGGACAAACTATTTTCCACCTCCAAAGAAATTAACACA

```

SUBSTITUTE SHEET (RULE 28)

FIG. 5

```

64 FF LSHLAVVNIA YACNTVPQMLVNLHPAKPISFAGCMTLDFLFLSFAHT 113
|||||:||||| .||||:||||| .|||||
1 FF LSHLAIVDIA YACNTVPQMLVNLDPVKPISYAGCMTQTFLELTFIT 50

```

1114 ECLLVMSYDRYVAICHPLRYFIINTWKVCITLGITSWTCGSLAMVHV 163
 |||||:||||| |||:| |.:|||..| |.:|:|
 51 ECLLVMSYDRYVAICHPLRYSAIMSWRVCSTMAVTSWIIGVLSLIHL 100

```

1164 SLILRLPFCGPREINHFFCEILSVRLACADTWLNQVVFECMFILVGP 213
| : | . | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1101 VLLLPLPFCVSQKVNHFCEITAILKLACADTHLNETMVLAGAVSVLVGP 150

```

LCLVLVSYSHILGGILRIQSGEGRRKAFSTCSSHLCVGLFFGSAIVMYM 263
:: ::|||. |||:|||:|||:|||:|||:|||:|||:|||:|||:|||:
FSSIWVSYACILGAILKIQSEEGQRKAFSTCSSHLCVGLFYGTAIMVTV 200

264 APKSRHP EEQKVLFILQFLS 285

1
 2
 3
 4
 5
 6
 7
 8
 9
 10
 11
 12
 13
 14
 15
 16
 17
 18
 19
 20
 21
 22
 23
 24
 25
 26
 27
 28
 29
 30
 31
 32
 33
 34
 35
 36
 37
 38
 39
 40
 41
 42
 43
 44
 45
 46
 47
 48
 49
 50
 51
 52
 53
 54
 55
 56
 57
 58
 59
 60
 61
 62
 63
 64
 65
 66
 67
 68
 69
 70
 71
 72
 73
 74
 75
 76
 77
 78
 79
 80
 81
 82
 83
 84
 85
 86
 87
 88
 89
 90
 91
 92
 93
 94
 95
 96
 97
 98
 99
 100
 101
 102
 103
 104
 105
 106
 107
 108
 109
 110
 111
 112
 113
 114
 115
 116
 117
 118
 119
 120
 121
 122
 123
 124
 125
 126
 127
 128
 129
 130
 131
 132
 133
 134
 135
 136
 137
 138
 139
 140
 141
 142
 143
 144
 145
 146
 147
 148
 149
 150
 151
 152
 153
 154
 155
 156
 157
 158
 159
 160
 161
 162
 163
 164
 165
 166
 167
 168
 169
 170
 171
 172
 173
 174
 175
 176
 177
 178
 179
 180
 181
 182
 183
 184
 185
 186
 187
 188
 189
 190
 191
 192
 193
 194
 195
 196
 197
 198
 199
 200
 201
 202
 203
 204
 205
 206
 207
 208
 209
 210
 211
 212
 213
 214
 215
 216
 217
 218
 219
 220
 221
 222
 223
 224
 225
 226
 227
 228
 229
 230
 231
 232
 233
 234
 235
 236
 237
 238
 239
 240
 241
 242
 243
 244
 245
 246
 247
 248
 249
 250
 251
 252
 253
 254
 255
 256
 257
 258
 259
 260
 261
 262
 263
 264
 265
 266
 267
 268
 269
 270
 271
 272
 273
 274
 275
 276
 277
 278
 279
 280
 281
 282
 283
 284
 285
 286
 287
 288
 289
 290
 291
 292
 293
 294
 295
 296
 297
 298
 299
 300
 301
 302
 303
 304
 305
 306
 307
 308
 309
 310
 311
 312
 313
 314
 315
 316
 317
 318
 319
 320
 321
 322
 323
 324
 325
 326
 327
 328
 329
 330
 331
 332
 333
 334
 335
 336
 337
 338
 339
 340
 341
 342
 343
 344
 345
 346
 347
 348
 349
 350
 351
 352
 353
 354
 355
 356
 357
 358
 359
 360
 361
 362
 363
 364
 365
 366
 367
 368
 369
 370
 371
 372
 373
 374
 375
 376
 377
 378
 379
 380
 381
 382
 383
 384
 385
 386
 387
 388
 389
 390
 391
 392
 393
 394
 395
 396
 397
 398
 399
 400
 401
 402
 403
 404
 405
 406
 407
 408
 409
 410
 411
 412
 413
 414
 415
 416
 417
 418
 419
 420
 421
 422
 423
 424
 425
 426
 427
 428
 429
 430
 431
 432
 433
 434
 435
 436
 437
 438
 439
 440
 441
 442
 443
 444
 445
 446
 447
 448
 449
 450
 451
 452
 453
 454
 455
 456
 457
 458
 459
 460
 461
 462
 463
 464
 465
 466
 467
 468
 469
 470
 471
 472
 473
 474
 475
 476
 477
 478
 479
 480
 481
 482
 483
 484
 485
 486
 487
 488
 489
 490
 491
 492
 493
 494
 495
 496
 497
 498
 499
 500
 501
 502
 503
 504
 505
 506
 507
 508
 509
 510
 511
 512
 513
 514
 515
 516
 517
 518
 519
 520
 521
 522
 523
 524
 525

201 GPRHGSPKEQKKYLLFHSLFN 222

FIG. 6A

```

1 MRPTWAGWLM.RWCCPPADTRCTFL..CSGRAAPQQAGAPHHPKYSLF 47
| |...:: :::::: ::::: : : . ::::: |...
1 MGPTSVP LVKAHRSSVDVYNYDIIVRHYNTGKLNISADKENSIKLTSV 50

48 PWIW.....KVRLPLPPLTTDVVPVRLTLRANLSAADLLRGRLPL 89
.:|:      .: .||.   ||... ..:: : ||. .||| | :..

51 VFILICCFIILENIFVLLTIWKTKKFHRPMYYFIGNLALSDLLAGVAYTA 100

90 PHVPHCPRTARLSLEGWF LRQGLLDTNLTASVATLLAIAVERHRSVMVAVQ 139
:  : | :|. .||||:| : .|.|||. .||||:|.|. .:: :.

101 NLLSGATTYKLTPAQWFLREGSMFVALSASFSLLAIAIERYTMLMKM 150

1140 LHSRLPRGRVVMLIVGVVVAALGLGLLP AHSWHCLCALDRSSRMAPLLSR 189
||.    . |:::|| ::||| . ||| || :|::||:::|| : ||. :

1151 LHNGSNFRFL LISACWVISLILGG LPIMGWN CISALS SCSSTVLPLYHK 200
```

MATCH WITH FIG. 6B

16 / 18

FIG. 6B

MATCH WITH FIG. 5A

```

190 SYLAVWALSSLLVFLLMVAVYTRIFFVRRRVQMA..EHVSCHPRYRET 237
    |:. . |::| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :|
201 HYILFCTTVFTLLLSIVILYCRYSLVTRSRRLTFRKNISKARSSE. 249
    |:. . |::| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :|
238 TLSLVKTVVILGAFVVCWTPGQVVLLD.GLGCESCNVLALEKYFLLLA 286
    .:| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :|
250 NVALLKTVIIVLSVFIACWAPLFILLLLDVGCKVKTCDILFRAEYFLVLA 299
    .| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :|
287 EPTSLVNAAVYSCRDAEMRRRTFRRLLLRVPFPPVHPRVCPLYILCPGRCQ 336
    .| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :|
300 VLNSGTNPPIIYTLTNKEMRRAFIR.....IMSCCKCP 331
    .| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :|
337 HSHHASRERPPTDGLHPLATLNYSGTR.....QATNPQPLMTCGC 376
    :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :| :|
332 SGDSAGKFKRPI.....IAGMEFSRSKSDNSSHPQKDEGDNPETIMSSG. 375

377 SWLNPTS 383
    :| :| :|
376 .NVNSSS 381

```

SUBSTITUTE SHEET (RULE 26)

17 / 18

```

1 MNTTVMQGFNRSKRCPKDTRIVQLVFPALYTVVFLTGILNLTALWVFW 50
  :|. | |. : | |... |. | :|. | :|. | :|. | :|. | :|. | :|. |
2 INSTSTQPPDES..CSQNLITQOIIPVLYCMVFIAGILNNGVSGWIFFY 49

51 IPSSSTFIIYLKNTLVADLIMTLMLPFKILSDSHLAPWQLRAFCRFSSV 100
  :|. | |. | |. | |. | :|. | :|. | :|. | :|. | :|. | :|. |
50 VPSSKSFIYLYLKNIVIAADFVMSLTFPFKILGDSGLGPWQLNVFVCRVSAV 99
101 IFYETMYVGIVLLGLIAFDRFLKIIRPLRNIFLKKPVWGKTVSIFIWFFW 150
  :|. | |. | |. | |. | :|. | :|. | :|. | :|. | :|. | :|. |
100 LFYVNMVYSIVFFGLISFDRYYKIVKPLWTSFIQSVSYSKLLSVIVWMLM 149

151 FFISLPNMILSNKEATPSSVKKCASLKGPLGLKWHQMNVNNICQFIFWTVF 200
  :|. | |. | |. | |. | :|. | :|. | :|. | :|. | :|. | :|. |
150 LLLAVPNIILTNQSVREVTQIKCIELKSELGRKWHKASNYIFVAIFWIVF 199
201 ILMLVFYVVIACK.YMILIESPKVRTEKTKSWKAKYLLSWLSSLCVL.. 247
  :|. | |. | |. | |. | :|. | :|. | :|. | :|. | :|. | :|. |
200 LLLIVFYTAITKKIFKSHLKSRRNSTSVKKKSSRNIFSI VFVFFVCFVPY 249

248 .....LHFISPEFHILTVKPTIRLTVDL.....KINCLLL. 277
  | :|. | |. | |. | |. | :|. | :|. | :|. | :|. | :|. |
250 HIARIPYTKSQTEAHYSCQSKEILRYMKEFTLLLSAANVCLDPIIYFFLC 299

278 .....KKQLSFWQQ..LTFVWIP 293
  | :|. | |. | |. | |. | :|. | :|. | :|. | :|. | :|. |
300 QPFREILCKKLHIPLKAQNDLDSRIK 326

```

FIG. 7

SUBSTITUTE SHEET (RULE 26)

18 / 18

6 SSHCFYNDSFKYTLGCMFSMVFLGVISNCVAIYIFICVLKVRNETTTYMINLAMSDDL 65
 SS+C DSEKYTLGCF+FSMVFLG+I+NCVAIYIF LKVRNETTTYM+NLA+SDLL
 3 SSNCSTEDSEFKYTLGCVFMSVFLGLIANCVAIYIFTFTLKVRNETTTYMLNLAISDLL 62

 66 FVFTLPFRIFYFTTRNWPFGDLLCKISVMLFYTNMYGSILFLTCISVDRFLAIVYPFKSK 125
 FVFTLPFRI+YF RNWPFGD+LCKISV LFYTNMYGSILFLTCISVDRFLAIV+PF+SK
 63 FVFTLPFRIYFVVRNWPFGDVLCKISVTLFYTNMYGSILFLTCISVDRFLAIVHPRSK 122

 126 TLRTKRNAKIVCTGVWLTVIGGSAPAVFVQSTHSQGNNAEACFENFPEATWKTLYLSRIV 185
 TLRTKRNA+IVC VW+TV+ GS PA F QST+ Q N CFENFPE+TWKTLYLSRIV
 123 TLRTKRNARIVCAVWITVLAGSTPASFFQSTNRQNTTEQRTCFENFPESTWKTLYLSRIV 182

 186 IFIEIVGFFIPLILNVTCSMVLKTLTKPVTLSRSKINKTKVLKMFVHLIIFCFCFVPY 245
 IFIEIVGFFIPLILNVTCS+MVL+TL KP+TLRS+K++K KVLKMFVHL+IFCFCFVPY
 183 IFIEIVGFFIPLILNVTCSMVLRLTLNKLPLTLRSNKLKSKKVLKMFVHLVIFCFCFVPY 242

 246 NINLILYSLVRTQTFVNCSVVAAVRTMYPITLCAVSNCCFDPVIVYFTSDTNSEFNKNE 305
 NI LILYSL+RTQT++NCSV AVRTMYP+TLCAVSNCCFDPVIVYFTSDTNSE +K +
 243 NITLILYSLMRTQTWINCSVVAVRTMYPVTLCIAVSNCCFDPVIVYFTSDTNSELDKKQ 302

 306 KL 307
 ++
 303 QV 304

FIG. 8

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04079

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07K 14/705, 16/28; C12N 15/12

US CL : 435/6, 69.1, 252.3, 320.1; 530/350, 388.22; 536/23.5, 24.31

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 69.1, 252.3, 320.1; 530/350, 388.22; 536/23.5, 24.31

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NATURE, Vol. 361, issued 28 January 1993, K. Raming et al., "Cloning and expression of odorant receptors", pages 353-356, see Figure 1 on page 354.	1-32
A	Proceedings of the National Academy of the Sciences USA, Vol. 89, issued October 1992, P. Nef et al., "Spatial pattern of receptor expression in the olfactory epithelium", pages 8948-8952, see Figure 1 on page 8949.	1-32



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"A" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

09 JUNE 1995

Date of mailing of the international search report

19 JUN 1995

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

JOHN D. ULM

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04079

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04079

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group I, claims 1-22 and 28-32, drawn to a nucleic acid encoding a putative receptor protein, the protein encoded thereby, and processes of use.

Group II, claim 23, drawn to an antibody which binds to a protein of Group I.

Group III, claims 24-27, drawn to a compound of undefined structure and chemical composition and methods of using that compound.

The inventions listed as Groups I, II and III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The protein and nucleic acid of Group I, the antibody of Group II and the compound of Group III are chemically and structurally unrelated and do not share a common technical feature. Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04079

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	The Journal of Biological Chemistry, Vol. 265, No. 16, issued 05 June 1990, T. Hla et al., "An Abundant Transcript Induced in Differentiating Human Endothelial Cells Encodes a Polypeptide with Structural Similarities to G-protein-coupled Receptors", pages 9308-9313, see Figure 2 on page 9311.	1-32
A	Proceedings of the National Academy of the Sciences USA, Vol. 90, issued May 1993, L. Rohrer et al., "Cloning and characterization of a fourth human somatostatin receptor", pages 4196-4200, see Figure 1 on page 4197.	1-32
A	FEBS LETTERS, Vol. 298, issued February 1992, N. Iwai et al., "Identification of two subtypes in the rat type I angiotensin II receptor", pages 257-260, see Figure 1 on page 258.	1-32
A	SCIENCE, Vol. 244, issued 05 May 1989, F. Libert et al., "Selective Amplification and Cloning of Four New Members of the G Protein-Coupled Receptor Family", pages 569-572, see entire document.	1-32